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Intensive agriculture to semi-natural grassland: evaluating changes in ecosystem service provision to help determine costs and benefits of agri- environment schemes

(Volume 1)

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Submitted for the degree of Doctor of Philosophy

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The University of Edinburgh

April 2013

Declaration

I hereby declare that this Thesis is my own work, except where otherwise stated, and has not been submitted in any form for another degree, diploma or professional qualification at any university or other institution

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Edinburgh, April 2013

Claire Alice Horrocks

Abstract

Intensive agriculture has led to an increase in production; however this has often coincided with a decline in the provision of other Ecosystem Services (ES). ES affected include those regulated by soil chemical, physical and biological properties such as biodiversity provision and the regulation of nutrient cycling, water quality and rates of greenhouse gas emissions. A growing awareness of the value of non-production ES to human health and wellbeing has encouraged the funding of agri-environment schemes in the UK, through which farmers receive funding to alter management practices to increase the provision of certain ES. One particular management change encouraged through agricultural payments is the creation of species rich grassland (SRG) on former intensively managed (IM) arable or grassland sites. Under these schemes farmers are required to carry out an extensification of management practices by reducing or ceasing fertiliser application, grazing and cultivation, or removing the existing crop or sward and sowing a specified seed mix of desired grassland species. Despite the commitment of substantial sums of money and land to extensification schemes, there has been little research into the extent to which they enhance provision of multiple ES and the potential for the legacy of intensive agriculture to limit ES provision and greatly reduce the value of extensification.

This study aimed to: 1) compare soil properties between sites remaining under intensive management and those that had undergone extensification; 2) relate soil properties to; fluxes of the greenhouse gas nitrous oxide (N_2O), plant diversity, soil microbial diversity and concentrations of nutrients in leachate from intensively and extensively managed sites in order to determine potential benefits of extensification.

Paired field plots were established on working farms in south east Scotland and at Rothamsted Research North Wyke in south west England. Each of the four plot pairs in Scotland consisted of a newly created SRG on former arable land and an adjacent IM plot. The SRG plots ranged in age from 3 to nine years old in 2010. Soil samples were collected from the Scottish sites twice yearly in 2010 and 2011, alongside regular measurements of N_2O fluxes from soil and assessment of plant diversity. At North Wyke four replicated SRG plots, forming part of an existing experiment on

former intensive grassland, were each paired with an IM plot. Soil samples were analysed for their chemical and physical properties and for the concentration of certain phospholipid derived fatty acids (PLFA) biomarkers to compare the composition and size of the soil microbial community. Soil N₂O fluxes and the nitrogen (N) and phosphorus (P) concentrations of soil water samples measured in 2010 and 2011. Results from more intensive N₂O flux measurements, conducted in 2012, were compared to model output from the UK-DNDC model to assess its potential for predicting changes in N₂O emissions following extensification.

No significant difference was found in any soil chemical or physical properties between paired IM and SRG plots in Scotland, although soil bulk density tended to be lower in the older SRG plots relative to the paired IM plots. Nitrous oxide emissions were low from all plots with only an occasional emissions peak being recorded and overall there was no significant effect of management intensity on soil N₂O fluxes. The UK-DNDC model outputs were generally of a similar order of magnitude but poorly correlated with measured N₂O fluxes and soil water and available N content. Botanical diversity was enhanced in the SRGs compared to the IM plots, though plant species were mostly of low conservation value and indicative of a high nutrient environment and the diversity of the SRG plots was low, compared to long-established semi-natural grassland elsewhere in Europe. Total soil PLFA concentration was significantly higher in the IM plots but the fungal concentration and the ratio of Gram positive:Gram negative bacteria were no greater in the SRG, suggesting it had begun to resemble long-term unimproved grassland. Despite limited success at obtaining soil water samples, at North Wyke concentrations of mineral N in soil water were lower from the SRG plots than the IM plots, although there were no consistent differences in total P or organic N concentrations, organic N contributed over 80% of the total N in soil water samples from the SRG plots.

This study has shown that the legacy of intensive agriculture continues to affect soil properties for at least 10 years following extensification. The results suggest that the potential for newly created SRGs to provide enhanced ES' could be limited and may not justify the reduction in productivity and the financial input associated with short-term extensification schemes.

Acknowledgements

I would like to thank the Natural Environment Research Council and the Scottish Environment Protection Agency for providing funding for my PhD. I would also like to thank The University of Edinburgh School of GeoSciences for accepting me into the University and allowing me to undertake my work and the farmers and land owners who allowed me to get in their way. Special thanks go to Rothamsted Research and the Biotechnology and Biological Sciences Research Council for providing valuable resources and expertise and for giving me opportunities and experiences I would not have got without their generosity.

From the Scottish Agricultural College I would like to thank Derek Robeson, without whose contacts the project would never have gotten started and Valentini Pappa for the use of her lab and equipment. From the University of Edinburgh I would like to thank my supervisors Kate Heal and Barbra Harvie for their support and guidance. Special thanks go to Rab Howard and Andy Gray for your endless patience and the many favours you did for me. Also I would like to thank my undergraduate field assistants from Edinburgh who provided company and an extra pairs of hands in the field, special thanks go to Kristina Simonaityte for the hours she spent in wind swept fields helping me identify grasses and for her incredible efficiency, also to Anthony Maire from Agro Paris Tech and Pierre Vincent from Avignon University for their hard work in the field and the lab helping measure soil chemical properties.

The greatest thanks however go to everyone at Rothamsted Research North Wyke for your kindness, generosity and friendship. Thanks to my supervisor Jenni Dungait and Roland Bol for saying yes to my request in the first place and for their on-going support and supervision, and to my other supervisors, Laura Cardenas, who has looked out for me throughout and Emma Pilgrim who helped me find my feet. Neil Donovan for his kindness, willingness to help, hard work and advice, without him most of my data would not exist. To Dan Dhanoa for statistical advice and technical assistance. To Deb Beaumont for providing the raw plant species data for the SRG plots. To everyone else at North Wyke, thanks for making me feel welcome and adopting me into you family, I can honestly say I will always be grateful to you all.

Finally thanks to my support network: the staff at the RD&E for taking care of me; the Prof. for giving me back confidence, all the friends I have made along the way and those who have been there since the start, to Roy for being a distraction and a therapy and to my mum for everything.

“Challenges are what make life interesting; overcoming them is what makes life meaningful”- Joshua J. Marine

“That which does not kill us makes us stronger”-Friedrich Nietzsche

“Give 100%. 110% is impossible. Only idiots recommend that.”- Ron Swanson

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List of abbreviations

AMSL – Above mean sea level

ANOVA – Analysis of variance

AWS – Automated weather station

BD – Bulk density

BDS- Bligh Dyer solvent

CAP- Common Agricultural Policy

CBD – Convention on BioDiversity

DCM – Dichloromethane

DEFRA – Department for Environment Food and Rural Affairs

DNDC - Denitrification-Decomposition

DON –Dissolved organic nitrogen

DWD - Drinking Water Directive

EAFRD – European Agricultural Fund for Rural Development

EC – European Commission

EI- Ellenberg indicator

EI-light - Ellenberg indicator value for light

EI-moisture - Ellenberg indicator value for moisture

EI-N - Ellenberg indicator value for nitrogen

EI-pH – Ellenberg indicator value for pH

ES – Ecosystem service

EU – European Union

FAME - Fatty acid derived methyl-ester

GC – Gas chromatograph

GC/MS - Gas chromatography – mass spectrometry

GHG - Greenhouse gas

G+ve bacteria– Gram positive bacteria

G-ve bacteria– Gram negative bacteria

HD - Habitats Directive

HPLC – High performance liquid chromatography

IGER - Institute of Grassland and Environmental Research

IICCP - Independent Climate Change project

IM - Intensively managed

IQ range - Inter quartile range

L-forbs – Leguminous forbs

MA – Millennium Ecosystem Assessment

MDmax - Mean daily maximum temperature

MDmin - Mean daily minimum temperature

MeOH - Methanol

Met. – Meteorological

mRNA-messenger ribonucleic acid

ND - Nitrates Directive

NL-forbs - Non-leguminous forbs

NW – NW

OM – Organic matter

Pi - Inorganic P

PLFA – Phospholipid fatty acid

PTFE -- polytetrafluoroethylene

SAC - Scottish Agricultural College

SD - Standard deviation

SDI - Shannon Diversity Index

SOC - Soil organic carbon

SRDP – Scotland Rural Development Programme

SRG - Species-rich grassland

TOrgN - Total organic nitrogen

TOxN - Total oxidised nitrogen

UK-DNDC – United Kingdom Denitrification-Decomposition model

UNFCCC – United Nations Framework Convention on Climate Change

WFD - Water Framework Directive

WFPS - Water filled pore space

WHO - World Health Organisation

Chapter 1 Introduction

This Chapter will introduce the concept of ecosystem services (ES)s and highlight the problems of providing certain services from intensively managed (IM) agricultural land. For the purpose of this study intensive management refers to the management of agricultural land (grassland or arable) to maximise production and is characterised by the use of fertiliser and often herbicides and pesticides and the use heavy machinery to apply these as well as to carry out other management activities such as ploughing, sowing and harvesting. An overview will be given of environmental policies and funding mechanisms encouraging farmers to convert intensively managed land to more extensive management, with particular focus on the European Community (EC). Next, detail will be provided on one particular land use change for which funding is provided to Scottish farmers, namely the creation of species-rich grassland (SRG) on former intensively managed sites. The importance of understanding the effect of this management change on ES provision in order to evaluate the benefits of such schemes will be emphasised. Finally the objectives and hypotheses of the research will be set out along with the structure of the thesis.

1.1 Introducing ecosystem services

Ecosystem Services (ES)s are defined as the benefits people obtain from ecosystems which can include financial benefits as well as benefits to health and wellbeing (Millennium Ecosystem Assessment (MA), 2003, they are often divided into provisioning, regulating, supporting and cultural services (see list below). The provision of many ES' is closely linked to soil and soil function (as reviewed by Haygarth and Ritz, 2009), and human land use and soil management have greatly altered both ecosystems and the services they provide worldwide. Generally increases in production of commodities, such as food, have been at the expense of environmental regulatory services (Turner et al. 2007; Foley et al., 2005), with two thirds of the ES' defined by the MA (2003) found to be in decline world-wide

ESs impacted by the soil condition and function (as discussed by Haygarth and Ritz (2009) include:

Provisioning services

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- Provisioning plant growth to support production of food, timber, fibre, fuel and bio-chemicals
- Provision of habitat to support biodiversity and genetic resources
- Providing a platform for supporting construction and infrastructure
- Providing water storage

Regulating services

- Regulation of water quality through filtering and buffering of rain water and supply through impact on hydrological flows
- Regulation of climate through enhanced carbon retention and reductions in ecosystem dis-services, including N₂O emissions
- Regulating rates of erosion

Supporting services

- Supporting efficient internal cycling and processing of nutrients.
- Supporting terrestrial primary production by vegetation

Cultural services

- Supporting environments with aesthetic, educational, spiritual and scientific value and that can serve as areas for tourism and recreation
- Maintaining an archaeological record of former civilisation

1.2 Impacts of intensive agriculture on ES provision

Intensive agricultural management practices, such as fertiliser, pesticide and herbicide use and the planting of high yielding crop varieties, have led to an increase in production for example by enabling dairy cow stocking rates in New Zealand to increase from 1.9 to 2.6 cows ha⁻¹ between 1991 and 2003 (Monaghan et al., 2005), in the UK grassland productivity has also been enhanced but this required an increase in

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average N fertiliser application rates from 15 to 130 kg N ha⁻¹yr⁻¹ between 1950 and 1980 (Van Der Meer and Van Uum-Van Lohuyzen, 1986). Crop production has also increased due to intensive management; between 1961 and 1999 the global crop yield per unit area increased by 106% but this required a 638% and 203% increase in nitrogen (N) and inorganic phosphorus (Pi) fertiliser use respectively (Green et al., 2005). Management to maximise production has been shown to cause a decline in other ES' (MA 2005); including the regulation of water quality and nutrient cycling and maintenance of biodiversity, whilst mixed effects of increased production have been reported on climate regulation (Pilgrim et al. 2010). The question of how to maximise provision of other ESs, whilst providing sufficient food to feed a growing global population, with increasing per capita consumption (Bennett and Balvanera, 2007; MA 2005) is a complex one. For example, organic farming practices, traditionally considered to enhance environmental ES provision, have been found to result in lower rates of harmful nutrient leaching and greenhouse gas emissions per unit area, but higher rates per unit product because productivity is reduced (Tuomisto et al., 2012). Furthermore, management aimed at enhancing provision of one service can often reduce provision of another (Bennett et al., 2009). For example the use of a slurry injection technique, to decrease NH₃ emissions, was found in some cases to lead to increases in soil N₂O emissions (Chadwick et al., 2011); this is one of many examples of so called 'pollution swapping' (Stevens and Quinton, 2009). Crop land and pasture occupy around 40% of the land surface; how we manage them to provide the optimal balance of ES' is becoming an issue of increasing global concern (Bennett and Balvanera 2007; Foley et al., 2005).

1.2.1 Impact of intensive agriculture on water quality

Nutrient enrichment of waters from fertiliser application can decrease water quality, with effects such as toxic algal blooms, reductions in fish populations and reduced amenity values of waterways (Carpenter et al. 1998). In many countries diffuse pollution from agriculture is the major cause of phosphorus (P) enrichment of surface waters (Kronvang et al., 2009), whilst estimates suggest that globally 23% of N applied to agricultural land could be lost to surface waters (Schlesinger, 2008). High nitrate (NO₃⁻) concentrations in drinking water can be particularly harmful to health as they increase the risk of methemoglobinemia in infants, currently 40,000 infants in

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the US are estimated to be exposed to drinking water nitrate concentrations exceeding the guideline of 50 mg L^{-1} recommended by the world health organisation via water sourced from privately owned wells (Knobeloch et al. 2000). Reductions in cases of methemoglobinemia have been attributed to careful regulating of public water supplies (Fewtrell L., 2004) however the closer of boreholes and in some cases necessary water treatment due to high NO_3^- concentrations are costly (Knapp, 2005) and (section 2.6)

1.2.2 Impact of intensive agriculture on climate regulation

Carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), three potent greenhouse gases (GHGs), are emitted from agricultural soils; these emissions have a significant impact on the global GHG budget and global warming potential (Johnson et al., 2007). Although increasing crop yields has the potential to increase carbon (C) sequestration in plant and microbial biomass (an ES) (Burney et al. 2009), it is estimated that a third of total C released to the atmosphere since 1850 has been the result of land use change such as deforestation to make way for agriculture (Houghton, 2003). Nitrous oxide is a potent greenhouse gas, with a global warming potential (GWP) 296 times that of CO_2 (molecule-for-molecule) and a lifetime of around 120 years in the atmosphere (Van Groenigen et al., 2010), its emission from agricultural soils can be seen as an ecosystem dis-service, and any action that reduces its emission either through increased N retention in soil or enhanced reduction of N_2O to N_2 (Chapter 2) is for the purpose of this study seen as an ES. The IPCC (2007) estimates that 42% of current global N_2O emissions are derived from agriculture through the use of nitrogenous fertiliser and cropping. In some countries this percentage is a lot higher; 78% of total N_2O emissions in the US and 63% in the European Union (EU) are estimated to be derived from agriculture (Johnson et al., 2007). Methane emissions from agriculture are often linked to enteric fermentation in livestock, however, when nutrient enriched soils are waterlogged, such as during rice cultivation, it can lead to high CH_4 emissions from soils (Mosier et al., 1998).

1.2.3 Impact of intensive agriculture on biodiversity maintenance

Clearance of natural vegetation for agriculture has led to the global loss of 50% of natural habitat on agriculturally usable land. Intensive agricultural practices are a

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major threat to biodiversity. Using birds as an indicator taxon for biodiversity, it has been estimated that in the developed world agriculture is responsible for the decline of 24%, of now threatened species and 33% of now near-threatened species (Green et al., 2005); in developing countries the contribution of agriculture is even greater.

Across Western Europe, plant species native to semi-natural habitats, such as extensively managed grassland, are threatened by an increase in management intensity to increase productivity. Bio-diverse, nutrient poor grasslands are being lost due to fertiliser and pesticide use (Isselstein et al., 2005; Walker et al., 2004). One study of 271 agricultural sites across six European countries found N input was significantly negatively correlated to plant species richness for both arable and grassland species (Kleijn et al., 2009). Cultivation and increases in nutrient availability in agricultural soils have also been shown to decrease the diversity of soil microbes (Oehl et al., 2003). Losses in the plant and microbial diversity have resulted in loss of key plant microbe interactions (Matson et al., 1997) vital for efficient ecosystem function. The maintenance of biodiversity enhances the provision of other vital ES' (Balmford et al., 2002; Baumgartner, 2007, Nielsen et al., 2011) for example by increasing productivity and efficiency in ecosystem function, providing a bank of genetic variability to increase ecosystem resilience, providing cultural and recreational services and maintaining a resource of organisms with current and potential direct human use.

1.3 International legislation to enhance global ES provision

With the increasing awareness of politicians and other policymakers of the threat posed to humans from the loss of non-production ESs, international agreements and protocols have been established which aim to encourage global cooperation in enhancing provision of these services. Global agreements, to which the UK is a signatory, include The United Nations Framework Convention on Climate Change (UNFCCC) and The Convention on Biodiversity (CBD). As a member state of the EU, the UK must also meet requirements of The Habitats Directive (HD) and the Water Framework Directive (WFD). In order to help meet the targets set out by these agreements, the EU specifically regulates agricultural practices and land use in all

member states through the Common Agricultural Policy (CAP), delivering payment to land managers through the European Agricultural Fund for Rural Development (EAFRD) to encourage environmentally sensitive farming.

1.3.1 The United Nations Framework Convention on Climate Change

The UNFCCC has been almost universally adopted, with 194 states and the EU having signed it (UNFCCC, 2012a). It recognizes that the problem of climate change is being exacerbated by anthropogenic GHG emissions and sets the broad aim of stabilizing GHG concentrations to prevent dangerous anthropogenic changes to global climate (UNFCCC, 2012b). Within the broad statements of the UNFCCC the Kyoto Protocol set out particular targets and commitments, which signature states were obliged to meet by 2012, and formed the basis for the Cancun Agreements, aimed at making countries set out legally binding commitments to reduce GHG emissions beyond 2012 (UNFCCC, 2012c). The significant role played by intensive agriculture in global GHG emissions (Section 1.1), means that meeting the goals of the UNFCCC will be heavily dependent on the future management of agricultural land.

1.3.2 The Convention on Biodiversity

The UK is also a signatory of the CBD, another international agreement with 168 signatories whose aims include the conservation of biological diversity (CBD, 2012a) and which sets goals under the Aichi Biodiversity Targets (CBD, 2012b). Where intensive agriculture has led to loss of biodiversity, signatories to the treaty will have to take targeted action to mitigate these losses and meet their obligations under the convention.

1.4 E.C. directives and agricultural subsidies

1.4.1 The Habitats Directive

The HD (HD, 92/43/EEC) aims to ensure the maintenance of biodiversity through the conservation of natural habitat. EU member states are required under Objective 2 of the directive “to conserve and restore biodiversity and ecosystem services in the wider EU countryside” and to pay particular attention to “extensive

farming...systems at risk of intensification...and design and implement measures to retain or restore conservation status” (EC, 2006). The importance of agriculture in contributing towards biodiversity protection is emphasised under Target 3 of the conclusions adopted following the EU 2020 biodiversity strategy meeting in 2011 (EC, 2011a).

1.4.2 E.U. directives concerning water quality

The Water Framework Directive (WFD, 2000/60/EC) was adopted by EU members and is a legal framework to protect and restore water quality throughout Europe. It requires an integrated approach, across entire river basins, to monitor and reduce the pollution of surface waters, groundwater and coastal waters from chemicals including nitrates, phosphates and pesticides from derived from agricultural activities. The aim is to achieve a ‘good ecological status’ (EC, 2008). Closely linked to and supporting the WFD, is the Nitrates Directive (ND, 91/676/EEC), which was implemented almost a decade earlier and requires all EU Member States to draw up action programmes to reduce the concentration of nitrates in surface water and groundwater. Again, particular mention is given to agriculture, which is responsible for over 50% of nitrogen discharged to surface waters (EC, 2010). Under the ND, codes of good agricultural practice have to be established by Member States to be implemented voluntarily by farmers. More specific action programmes were developed for areas designated as Nitrate Vulnerable Zones (NVZs), within which implementation of action plans by farmers is compulsory (EC, 2010). The Ground Water Directive (GWD, 2006/118/EC) addresses the requirements of the WFD relating to groundwater, and sets specific targets to limit pollution of groundwater by nitrates and pesticides by 2015. It includes measures to regulate diffuse sources such as agriculture (EC, 2011b). The Drinking Water Directive (DWD, 98/83/EC) is another EU requirement with implications for agricultural management. It requires drinking water to be of a certain standard to protect human health. EU member states must monitor water quality to ensure the maximum nitrate concentration for drinking water of 50 mg L^{-1} , set by the World Health Organisation (WHO; WHO, 2011) is not exceeded (EC, 2010). Nitrate removal from water to meet the DWD requirements was estimated to cost at least £16 million (\$24.4) million a year between 1990 and 1996 in the UK alone (Pretty et al., 2000), with the total costs of freshwater

eutrophication in England and Wales estimated at between £75 million and £114.3 million pounds per year (Pretty et al., 2002).

1.4.3 The Common Agricultural Policy

In the EU direct support and subsidies are provided to farmers to encourage them to carry out specific farming, environmental and development activities through the Common Agricultural Policy (CAP). The CAP consists of two pillars of funding. The second pillar, which accounts for nearly 25% of CAP spending, provides funding for rural development initiatives (DEFRA, 2011) through the European Agricultural Fund for Rural Development (EAFRD). Included in the activities funded by the EAFRD are those aimed at enhancing the environmental value of land (agri-environment schemes), such as the extensification of agricultural management via the ceasing of fertiliser applications (EC, 2009).

Under the agreement of the CAP all EU Member States must devise a Rural Development Programme (EC, 2005). In Scotland EAFRD funding is distributed through the Scotland Rural Development Programme (SRDP). The programme received 680 million euros from EAFRD, along with additional funding from the Scottish Government, to spend between 2008 and 2013 (Scottish Executive, 2011). The SRDP has three ‘axes’ or areas of focus, of which the second axis is concerned with ‘improving the environment and the countryside’ (Scottish Executive, 2009). Money is partly distributed under the SRDP through rural development contracts (RDCs), for which competitive applications are submitted (Scottish Executive, 2007). Land managers apply for funding for specific options, detailed under the RDCs rural priorities list for each Scottish region. Among the available RDC options are those encouraging the restoration of semi-natural grassland on former intensive agricultural land. These options are available under both ‘biodiversity and landscape’ and ‘water quality’ regional priority categories (Scottish Executive, 2009). The listing of SRG creation under these priorities highlights the current assumption by policy makers that created SRG sites will provide multiple benefits in terms of ES provision.

1.4.4 Habitat banking

A final potential driver for land use change is habitat banking, which has been suggested as a means of offsetting environmental impacts of development. This is done by replacing habitat lost to development through restoration of an equivalent area of the same habitat elsewhere. This bank of restored habitat in a region would then be seen to compensate for losses elsewhere (Briggs et al., 2009).

1.5 Calculating the costs and benefits of agri-environment schemes

1.5.1 The need to understand and maximise ES provision from agri-environment schemes

Agri-environment schemes require the removal of land from maximum intensity production, referred to here after as extensification. Between 2008 and the end of March 2012, £2,166,244 had been spent on the creation of species rich grassland schemes and £727,169 had been spent on funding arable reversion to grassland in Scotland (Scottish Executive, 2012). In England by the end of 2012 there were over 80,000 ha of created or restored grassland (Wilson et al., 2013).

In the future, pressures on available land worldwide are set to increase due to: a predicted global population rise from 6.7 billion in 2008 to 10 billion by 2100; increased demand for bio-fuels; and increased meat consumption in the developing world (Lal, 2008). The CAP represents 40% of the EU's budget and an increasing proportion of this is going towards rural development initiatives (EC, 2012). It is important that this money is well spent and that the enhancement in environmental ES provision following extensification can justify both the loss of production and the cost of the financial subsidy awarded to farmers, this concept is summarized graphically in Figure 1.1. The environmental cost of land use displacement should also be considered in this calculation; production lost through measures aimed at reducing environmental harm in one location is often replaced by enhanced production elsewhere, which can result in increased environmental harm in the new location (Lambin, E.F. and Meyfroidt, P. 2011).

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The trading of GHG emissions allowances is currently used to cap GHG emissions from energy producing and industrial activities by signatories of the Kyoto protocol, one such trading scheme is the European Union Emissions Trading Scheme (EU ETS). The potential to extend these schemes in the future to include other sectors, is emphasised in the protocol and the adaptation of existing schemes such as the EU ETS to include emissions from agriculture is currently being debated (Perez Dominguez et al., 2009; Svendsen, G.T. and Brandt, U.S. 2011). This could provide a further financial incentive in the future for enhancing climate regulation services from agricultural land. In addition there have been calls to introduce payments to farmers for other ES provided by the land they manage (Swinton et al., 2007). The effective implementation of payments for ecosystem services (PES) schemes and the ability to optimise CAP payments and land use all require a detailed understanding of the effect of land use change on multiple ES' (Antle et al., 2003).

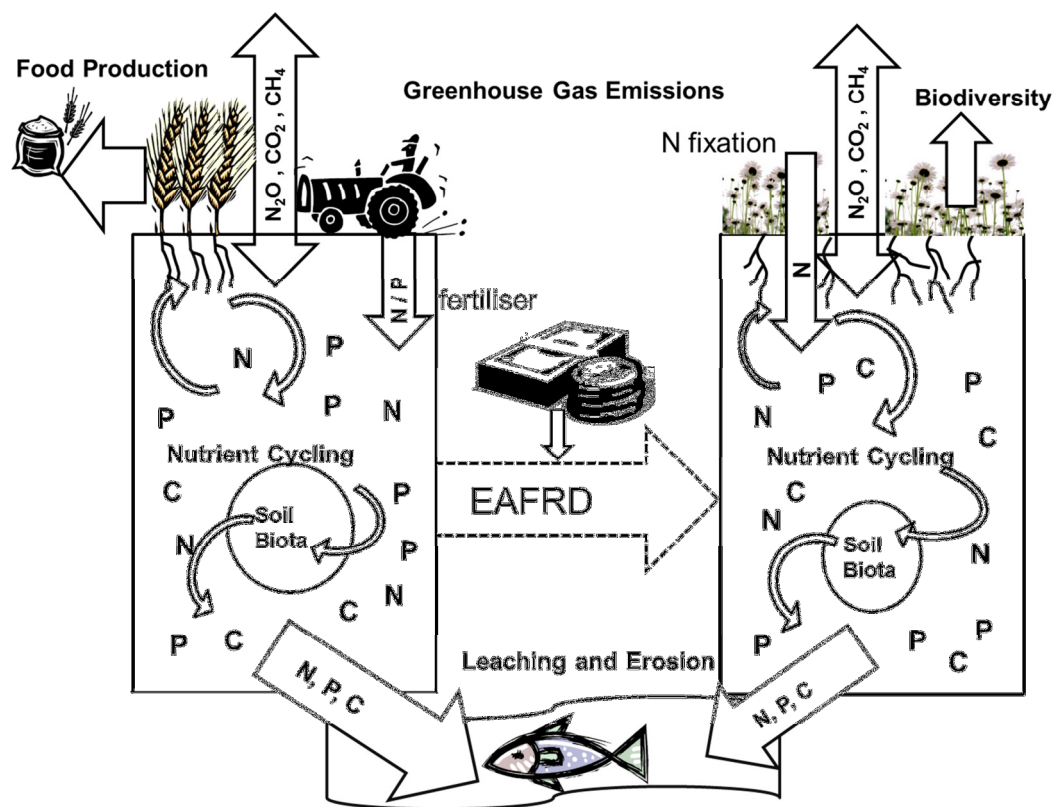


Figure 1.1 Conceptual diagram summarizing Ecosystem Services potentially supported by soil under intensive management (left hand side) and following conversion to extensively managed grassland under an agri-environment scheme (right hand side). The figure aims to

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highlightsthe importance of soil chemistry (N P and C concentration) and the soil biota and plant community in regulating nutrient cycling and potential losses under both systems.

1.5.2 Uncertainties and knowledge gaps preventing a complete assessment of ES provision following extensification

There is a growing understanding of the potential for land-use legacies to continue to affect ecosystem service provision for many decades (Foster et al., 2003). The extent to which changes in ES provision resulting from intensive management are reversible if agri-environment schemes are implemented, is a critical area of research with consequences for future global sustainability (Bennett and Balvanera 2007). The potential for an agricultural legacy to limit ES provision following extensification is reviewed in Chapter 2.

Despite the importance of ensuring agri-environment schemes are an effective use of land and money, evaluations of their efficacy in providing the desired ecosystem service have often been lacking (Kleijn and Sutherland, 2003). The lack of evidence as to the effectiveness of interventions designed to enhance ES provision has been highlighted in the literature e.g. by (Carpenter et al., 2009). Where evaluation of agri-environment schemes has occurred the tendency has been to focus on the provision of a single ES, despite recent calls for more multidisciplinary research looking at optimising provision of multiple ES' for multiple stakeholders (Bennett and Balvanera 2007).

It is not always possible to assign a direct monetary value to an ES, so balancing the cost of a reduction in one service against the gains in another can be difficult. However concepts for valuing ES' are being developed as tools to help decision makers (Farber et al., 2002).

1.6 Research objectives

The objectives of the research were to:

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1) Compare the potential for ES provision from species rich grasslands (SRGs) that have been created within the last 10 years with that of sites remaining under intensive management (IM sites). At the studied SRG sites:

- All fertiliser applications had ceased
- The original crop had been ploughed up prior to the sowing of a grassland seed mix.
- Management activities such as low density grazing and / or cutting were on-going

The study aimed to determine the extent to which ES provision from such sites is limited by the legacy of the former intensive management on key soil properties.

3) Following identification of knowledge gaps the decision was made to focus on comparing soil chemical and physical properties of extensively and intensively managed sites and to use the following as indicators of ES provision:

- Soil chemical and physical properties
- Emissions of the greenhouse gas nitrous oxide;
- Soil microbial and plant diversity;
- Mineral loss in soil leachate;

4) Provide data that can be used in assessing the benefit of converting IM sites to extensively managed SRGs.

5) Identify factors, such as time since establishment and nature of the former intensive management, with the potential to enhance ES provision from SRGs and use this to provide recommendations for future research and evidence based agricultural policy.

1.7 Hypotheses

The research objectives were addressed through the following specific hypotheses.

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1. Intensive agriculture has long lasting effects on some soil chemical, physical and biological properties that continue to limit the potential for ES provision at SRG sites younger than 10 years old (considers created SRGs that have been entered into a maximum of two 5-year commitment periods under the SRDP)
 - 1.1 SRGs created at sites with a history of organic N addition, will have high residual concentrations of total soil N and C, similar to IM fields..
 - 1.2 Legacy effects of intensive agriculture on the soil C:N ratio, the soil biota, and plant-microbe interactions will maintain high rates of net mineralisation in SRG plots and similar NO_3^- concentrations similar to the IM plots.
 - 1.3 SRGs will have high soil P concentrations, equal or similar to IM fields, due to accumulation of recalcitrant P forms under intensive management, gradual dissolution of P will maintain similar concentrations of available P in IM and SRG plots
 - 1.4 Soil bulk density (BD) of SRGs will be similar to that of IM fields due to slow rates of recovery of soil structure following cultivation
2. Some soil properties relevant ES provision at SRG sites will change during the first 10 years following conversion
 - 2.1 Soil organic matter (SOM) concentration will increase at SRG sites following conversion from IM, due to an increased return of organic matter to soil, which accumulates over time.
 - 2.2 Over the first 10 years increased N fixation and higher returns of plant matter to soil in the SRGs will increase total soil N.
 - 2.3 Increased OM addition to SRG soils will increase the supply of organic acids to the soil leading to pH decrease. will lead to a gradual decrease in soil pH particularly of SRG plots.
3. High soil nutrient concentration relative to long established species rich grasslands and a limited seed bank will limit the establishment of desired

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plant species with few species from sown seed mixes establishing. Land cover at SRG sites will be dominated by species indicative of fertile soils.

- 4 The size and composition of the microbial community at SRG sites younger than 10 years old will be similar to IM sites due to the slow rate of changes in the soil microbial community following conversion to SRG.
- 5 No or limited decrease in soil N content and little change to the soil microbe community will lead to similar background N₂O fluxes from both IM and SRG sites.
- 6 High residual soil P and sustained N availability will result in substantial concentrations of dissolved N and P in soil water samples from SRG and IM sites.

1.8 Thesis structure

Chapter 2 will review the literature, assessing the potential for an agricultural legacy to limit ES provision following extensification and highlighting areas of uncertainty. These areas of uncertainty will then be addressed in the rest of the thesis.

Chapter 3 will describe two sets of field sites, one set in South East Scotland and one in South West England, at which field work was conducted. The methods used to test the hypotheses posed in Chapter 1 will be presented. For each type of measurement the methodology used at the Scottish sites will be presented followed by that used at the English sites.

Chapters 4 and 5 will present and discuss the results from the data collection. Chapter 4 will present all the results from the Scottish sites and Chapter 5 will present the results from the English sites. As rainfall and temperature can have a strong bearing on soil processes and function Chapters 4 and 5 will start with a section comparing the climate at the sites during the experimental period, with the average climate conditions for the area. Both chapters will then be divided into sections describing the methods of data analysis and the results from each set of field measurements in turn and in the following order (note that not all measurements were made for both sites; further details are provided in Chapter 3):

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1. *Key soil chemical and physical properties* - these can influence ES provision potential.
2. *Plant species surveys*- as well as being a key ES in itself, biodiversity and plant species identity can regulate ecosystem function and therefore the provision of other key ESs.
3. *Phospholipid fatty acid (PLFA) analysis of the soil microbial community composition*- the soil micro-biota is strongly regulated by plant-soil feedbacks. The microbial community drives soil nutrient cycles and rates of microbial processes regulating for example GHG emission and nutrient leaching rates.
4. *Nitrous oxide (N_2O) flux measurements* – since N_2O is a potent GHG, measuring fluxes from soil provides a direct assessment of ES provision from a site.
5. *Comparisons of N_2O flux measurements with UK-DNDC modelled fluxes*- using the process-based UK-DNDC model aids an understanding of the factors regulating N_2O emissions. The ability to model N_2O fluxes following management changes could support future decision making.
6. *Measurements of N and P concentrations in solution in soil water*- this provides an indication of the water quality regulation potential of a site.

Following the presentation of the results from each set of measurements Chapters 4 and 5 will then contain a section, discussing the e in the context of findings from other studies.

Chapter 6 will discuss the combined data set and the extent to which it supports the hypotheses laid out in Chapter 1

Chapter 7 will conclude with the overall findings of the study, relate these to the original objectives and suggest further work.

Chapter 2 Review of the literature: The legacy of intensive agriculture on ES provision potential.

One potential, and apparently overlooked, limitation of the creation of SRGs under agri-environment schemes, is that the effects of intensive agricultural management on soil N, P and C content (Booth et al., 2005; Hart et al., 1994), and the soil biota (Foster et al., 2003; McLauchlan, 2006), may continue to affect soil nutrient cycling and nutrient loss. Nutrient loss pathways of particular concern are N and P in leachate and associated with sediment in runoff, as well as gaseous emissions of nitrogenous compounds, including the potent greenhouse gas nitrous oxide (N₂O). Numerous studies have examined the effects of different agricultural land uses and management practices on soil nutrient content, cycling and leachate. However the author has found no studies that specifically address the potential for the legacy of intensive management to lead to significant and sustained nutrient losses following extensification. This chapter aims to assess the evidence for potentially significant nutrient loss from land following the cessation of intensive agricultural management. Evidence was compiled worldwide from field studies of soil nutrient content, cycling and losses on former agricultural land across a range of soil types and management practices. Before-After/Control-Impact (BACI; Stewart-Oaten et al., 1986) experiments to investigate the effect on soil nutrient cycling of change from intensive to extensive agricultural management are not well-represented in the literature. Therefore, the majority of studies examined are of former intensively managed land, in which increasing time since abandonment is used to identify likely changes to ES provision. These studies have been reviewed in order to identify:

1. How the legacy of intensive agriculture could impact on soil chemical properties including the concentration and type of N, P and C compounds.
2. The vulnerability of soil N and P to loss following cessation of intensive management.
3. The legacy of intensive agriculture and impact of land use change on the above and below ground microbes and flora; and how this may affect the

potential ES provision from agri-environment sites and the extent to which the desired community represents that of long-established species rich grasslands.

4. The legacy of intensive agriculture on soil physical properties and how this might impact on ES service provision from created SRGs.
5. The efficacy of current methods aimed at reducing the legacy effect of intensive agriculture to maximise ES provision from agri-environment scheme sites.
6. The costs of sustained high rates of nutrient loss from created SRGs.

2.1 Effects of agricultural practices on soil N, P and C content

Agricultural practices regulate the size of soil nutrient pools, which are largely determined by the rates of transformation processes in the soil (illustrated for P and N in Figure 2.1 and Figure 2.2). The effects of agriculture on soil total N and C concentrations can still be observed over 50 years after the cessation of intensive agriculture (Kopecký and Vojta, 2009). Indirect evidence of elevated soil nutrient levels at sites formerly under intensive agricultural management is provided by the limited success of many attempts to restore species-rich lowland grassland on former intensively managed agricultural sites in Europe. This is often attributed to high soil N and particularly P concentrations preventing the establishment of the desired, high diversity ecosystems (Smith, R.S. et al., 2003; Smits et al., 2008; Tallowin and Smith, 2001), and instead leading to domination by a few grass species characteristic of high nutrient environments (Pywell et al., 2007). More species-rich grassland communities develop at former intensive agricultural sites, where bare ground and high precipitation cause nutrient leaching (Walker et al., 2004) and a reduction in soil nutrient content. In the remainder of this section the effects are examined of intensive agricultural management and its legacy for concentrations, pools and fractionation of soil P, N and C, respectively

2.1.1 Effects on soil P content

Globally rates of soil P accumulation increased from 11 to 15 Tg P yr⁻¹ from 1970 to 2000 (Bouwman et al., 2009) primarily due to increased fertiliser and animal manure

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application to remedy the often limited availability of soil P. Furthermore, since typical manure has a lower N:P ratio than is required by plants, application of manure up to allowable rates of N addition can result in soil P accumulation (Smith et al., 1998a). Another cause of P accumulation in agricultural soils is the low susceptibility of added P to leaching. In alkaline soils P precipitates as various calcium phosphates. In neutral and acidic soils orthophosphate ions (PO_4^{3-}) are readily adsorbed onto clay minerals and precipitate with aluminium (Al) and iron (Fe) oxides to form insoluble phosphates (Leinweber et al., 2002).

The extent to which P accumulates in soil under intensive agricultural management varies greatly between sites depending on the land use and management practices. Where P inputs are low, removal in crops or by grazing can result in little change or even a reduction in soil P content under intensive management (Sigua et al., 2006).

Where P addition exceeds removal by crops, soil P accumulation occurs and can be long-lasting. In a comparison of soil nutrient pools under oak forest planted between 1920 and 1960, it was found that the total soil P concentration at 10-30 cm depth was significantly greater in the sites which had been formerly cultivated compared to those which had been continuously forested for at least 300 years. The total soil P pool at 10-30 cm depth was still significantly higher in the formerly cultivated soils, even 40-80 years after cultivation had ceased (Falkengren-Grerup et al., 2006).

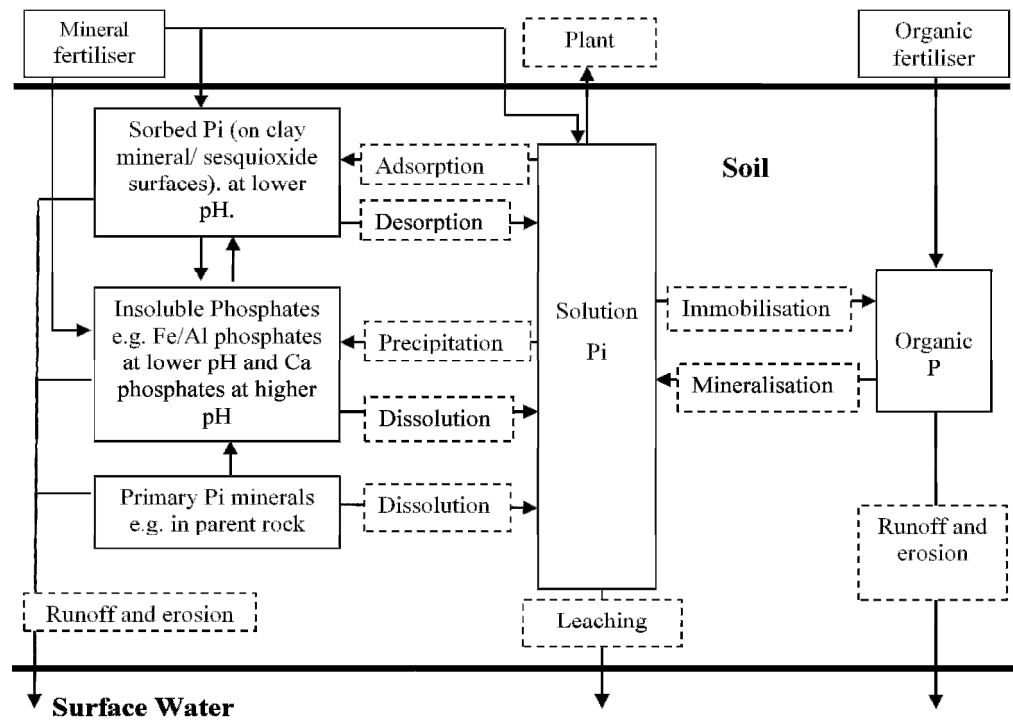


Figure 2.1 The P cycle in agricultural systems, showing the routes of P addition to and loss from soils, along with the key transformation processes between different soil P pools. Adapted from Campbell and Edwards (2001).

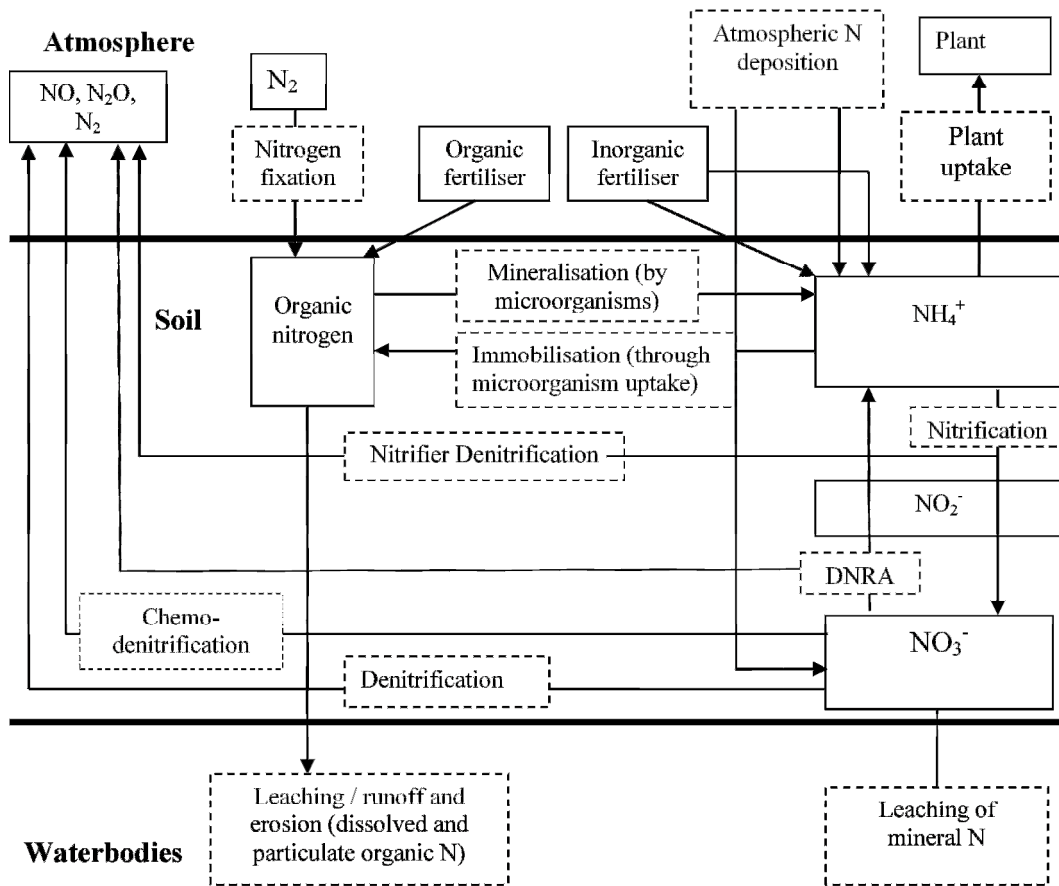


Figure 2.2 The nitrogen cycle in agricultural systems showing the routes of N addition to and loss from soils, along with the key transformation processes between different soil N pools. Adapted from (Hatch et al., 2002). DNRA is dissimilatory nitrate reduction to ammonium.

2.1.2 Effects of intensive agriculture on soil N content

N can be added to soil in fertilisers, through the actions of N-fixing bacteria, and from atmospheric N deposition (Jefferies and Maron, 1997) (Figure 2.2). As with P, crop removal reduces N recycling through decomposition of plant OM. However, unlike P, mineral N in soil in the form of nitrate (NO_3^-) and nitrite (NO_2^-) is readily soluble and susceptible to leaching. Ammonium (NH_4^+) is often tightly bound by clay particles but is readily converted by nitrifying micro-organisms to NO_2^- (Hatch et al., 2002).

Agricultural practices, such as tillage, can result in decline in SOM and associated soil N losses in leachate (Knops and Tilman, 2000) by reducing the physical protection of N in the soil, increasing soil temperatures, decreasing soil water holding capacity and increasing the activity of enzymes important in N mineralisation. As a result soil N concentrations can decline under intensive management. The impact of intensive management practices on soil N concentration can vary with soil texture. In a comparison of uncultivated Canadian prairies and former prairie sites that had been cultivated for 65-70 years, the decrease in total soil N concentration under cultivation was found to be greatest and occur to a greater depth in sandy soils (Tiessen et al. 1982). Further evidence of the soil N losses from cultivated sandy soils is provided by a chronosequence study in Minnesota, USA. Fields abandoned in 1927-1982 were compared with adjacent never farmed sites, it was estimated that agricultural practices lead to the loss of 75% of the soil total N pool (Knops and Tilman, 2000).

At other sites, in contrast, soil N accumulation has been reported under intensive agricultural management. In Massachusetts, USA, the total soil N concentration was significantly higher on land cultivated 90-120 years previously compared to permanently forested plots (Compton and Boone, 2000). Accumulation of soil N under agriculture has been linked to manure application and is attributed to the difference in the ratio of nutrients supplied in manure compared to the ratio of nutrient removal by common crops (Edmeades, 2003) and to high levels of manure spreading in some intensive livestock systems. For example, in a semi-arid environment in Alberta, Canada, soil N and P remained significantly elevated on

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manured plots compared with non-fertilised control plots for 16 years after manuring ceased, even under continued cultivation (Indraratne et al., 2009). An exponential decay model fitted to these data showed that it could take between 17 and 41 years before total N concentration in the surface soil decreases to that prior to manure addition, whilst extractable NO_3^- concentrations in the whole soil profile (0-150 cm depth) could take 182-297 years to recover. The rate of recovery was dependent on the amount of nutrients that had accumulated and on rainfall which affected nitrification rates and leaching loss. Soil N concentration reached pre-manure application levels faster than soil P concentration, due to the low N:P ratio of the manure applied and the more rapid loss of N through gaseous emissions and leaching.

2.1.3 Effects on soil C content and the implications for N and P cycling

Agriculture can also impact on soil organic carbon (SOC) content, which can potentially influence soil N and P cycling through effects on soil moisture storage, structure and microbial activity. The traditional view has been that SOC decreases under cultivation due to crop removal (Tiessen et al., 1982) but the addition of excessive manure can lead to increased SOC (McLauchlan, 2006), often accompanied by increases in soil total organic N. Any fertiliser application has the potential to increase SOC content due to greater crop yields, augmenting the quantity of OM returned to the soil in crop residue, although SOC increases have been found to be higher where organic fertiliser (manure) is used (Haynes and Naidu, 1998).

Any change in the C:N ratio in soils due to agriculture can affect the rate of microbial metabolic processes involved in both the immobilisation and mineralisation of N. In soils with a higher labile OM content and a greater C:N ratio, rates of microbial N immobilisation are enhanced, resulting in more rapid stabilisation of the readily mobile forms of mineral N, including NO_3^- , and a reduced rate of N loss (Barrett and Burke, 2000).

The effects of agriculture on soil C:N ratio can continue to impact on N cycling many years after agricultural abandonment. Nitrification rates in soil were reported to be higher at some sites 50 years after agricultural abandonment compared to plots that had never been cultivated (Compton and Boone, 2000). This is attributed to the

lower soil C at the formerly cultivated sites causing a decreased C:N ratio and hence decreased N immobilisation. Similarly at sites which had been uncultivated since the 1st century AD, Dupouey et al. (2002) recorded a lower C:N ratio and hence greater net N mineralisation in locations where cultivation and manure application had been the most intense. Lower rates of N immobilisation in former intensively managed agricultural soils could encourage greater rates of N loss, as more soil N remains in labile forms that are vulnerable to loss through leaching and gaseous emissions (Haynes and Naidu, 1998).

2.2 Vulnerability of accumulated soil N and P to loss in solution and through erosion

It is important to consider the vulnerability of any accumulated P and N to loss after intensive management ceases as some chemical forms are more vulnerable to loss than others.

2.2.1 Vulnerability of accumulated soil N and P to loss in solution

P accumulates in soil as different chemical fractions, some of which are more labile and vulnerable to leaching than others. The processes influencing the mobility of soil P vary with soil physio-chemical properties (Blake et al., 2003). Inorganic P (Pi) generally has a lower vulnerability to loss in solution compared to inorganic N, since it occurs mainly as phosphates (PO_4^{3-}), principally in insoluble calcium (Ca), Fe or Al phosphates or tightly adsorbed onto the surface of soil mineral or OM (Figure 2.1) However adsorbed Pi can desorb gradually from these sites and enter soil solution (Vu et al., 2010), becoming vulnerable to loss via leaching. A dynamic equilibrium has been identified between adsorbed Pi and Pi in soil solution, such that removal of Pi from solution by plant uptake or leaching, results in further desorption (Koopmans et al., 2004; Yli-Halla, et al., 2002). The positive association reported between soil Pi content and Pi concentration in soil solution, means that the risk of leaching of Pi increases with soil Pi content. The relationship is only apparent above a critical available soil P content (Heckrath et al., 1995), when P saturation of tightly sorbing clay sites occurs. This results in greater Pi sorption to weaker binding sites on the surface of aggregates, from where it is more readily released to solution. However, care is required when assessing the potential for P losses from agricultural soils

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based on the results of studies involving sampling of water from field drains (e.g., Heckrath et al., 1995). Higher losses of soluble Pi could occur from sites with artificial drainage, compared to similar sites without artificial drainage, due to a longer residence time of soil solution in un-drained soils, resulting in re-adsorption of Pi mobilised from enriched surface soils in less enriched sub surface soils (Blake et al., 2000).

Where soil N does accumulate under intensive agriculture, it could be gradually lost following extensification. The application of manure has been linked to an increase in soil organic N concentration (Section 2.1.2). Previously mineral N has been the focus of studies investigating the risk of N leaching from agricultural soils; however organic N can be vulnerable to loss by leaching, as many organic N-containing compounds are soluble. Soluble organic nitrogen comprises 40-50% of the soil total soluble N pool in arable soils in England with a wide range of textures (Murphy et al., 2000). A review of 16 field studies across a diverse range of agricultural systems showed that DON accounted for a mean of 26% of annual total soluble N mass loss, although this figure varied greatly from one to 74% (van Kessel et al., 2009). The main factors suggested to enhance DON leaching in agricultural soils were: i) high precipitation/irrigation events, especially after dry periods; ii) high N input; iii) high animal stocking rates; iv) sandy soil texture; and v) the presence of legumes. Once leached DON can have significant ecological impacts and adversely affect the ecology of rivers and estuaries (Jurgensone and Aigars, 2012; Seitzinger and Sanders, 1997; Vitousek et al., 1997). Organic N can also be mineralised readily by soil microorganisms to NH_4^+ and subsequently converted via nitrification to NO_3^- , which is highly soluble and vulnerable to loss in solution.

Alternatively if soil N is left depleted following intensive management, gradual accumulation through enhanced nitrogen fixation following extensification, could contribute to increasing DON losses over time from agri-environment scheme sites.

2.2.2 Vulnerability of accumulated soil N and P to loss through erosion

In addition to losses of P and N compounds in solution, more recalcitrant N and P compounds, typically seen as less mobile within the soil, can be lost through soil erosion when bound to fine sediment and colloidal material. Despite grasslands

typically being perceived as having a low erosion vulnerability, it has been shown that important erosion processes, for example the transfer of very fine colloids, do occur in grasslands making them a potentially significant source of nutrients to surface waters. Particularly significant quantities of P can be transported to surface waters, during high energy rainfall events (Heathwaite et al., 2005; Withers and Jarvie, 2008, Dungait et al., 2012). Although, even in the absence of high intensity rainfall, erosion processes can lead to nutrient loss (Bilotta et al., 2007). Kleinman et al. (2011) identified erosion processes as the largest contributor to the loss of P which has accumulated from past fertiliser additions, or ‘legacy P’.

Any legacy of intensive agricultural practices on the risk of soil erosion will also impact on soil N and P losses. Agricultural practices such as tillage can increase the rate of soil erosion due to increased compaction, decreased infiltration and a decrease in soil OM concentration (Section 2.1.3) (Hussain et al., 1999; McLauchlan, 2006), which is important in maintaining aggregate stability.

2.3 The effect of an intensive agricultural legacy and a change in management on the soil biota and the possible impact on soil nutrient cycling

2.3.1 The legacy of intensive agriculture on the soil biota

The soil microbial community has a vital and complex role to play in regulating nutrient cycling and loss processes and is itself regulated by soil chemical properties (Bardgett, 2005). Several studies have shown that the composition of soil bacterial communities controls denitrification processes and gaseous N fluxes from soil (Cavigelli and Robertson, 2000; Morales et al., 2010), with differences in the community composition reported between conventional and low intensity managed agricultural fields. The effect of the soil microbial community composition in controlling other nutrient cycling processes, such as nitrification and N fixation, which are carried out by a narrower set of microbial organisms has also been demonstrated (Schimel et al., 2005). In contrast, other key N cycling processes, such as N mineralisation and immobilisation, which follow the same conversion pathways across a diverse group of microbes, have typically been believed to be insensitive to

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changes in microbial community composition. This view was challenged by Schimel et al. (2005), who suggested that individual steps in N mineralisation / immobilisation, such as depolymerisation of specific organic compounds by extracellular enzymes, are carried out by a much narrower group of organisms. Consequently small changes in the soil microbial community can affect the rate of N cycling even through seemingly universal pathways. Nevertheless, changes in the soil microbial community have not always been shown to affect nutrient cycling (Donnison et al., 2000). Dandie et al. (2008) showed that N₂O emissions and denitrification rates for arable plots in New Brunswick, Canada, were not coupled to abundances of denitrifying bacteria.

Through practices such as lime and fertiliser application (Donnison et al., 2000) and tillage (Young and Ritz, 2000), agriculture can have significant effects on the soil microbial community. Changes in the soil biota and associated soil properties and ecosystem functioning can remain for many years after intensive management ceases (Liiri et al., 2012). For example Buckley and Schmidt (2001) showed that the structure of the microbial community, including organisms responsible for key nutrient cycling processes such as denitrifiers, was similar in active arable plots and plots abandoned over seven years previously, but significantly different in never cultivated sites.

Many arbuscular mycorrhizal fungi species, have a vital role in enhancing plant nutrient uptake in natural ecosystems (see section 2.3.4) but are not found in the most productive agricultural systems (Oehl et al., 2004). It is suggested that high soil mineral N and P content, along with frequent tillage under intensive agricultural management, limits fungal growth, particularly of mycorrhizal fungi (Bittman et al., 2005). Hence the abandonment or extensification of agricultural management typically increases the fungal:bacterial biomass ratio in soils (Bardgett and McAlister, 1999; van der Wal et al. 2006). However, after an initial increase in soil fungal biomass in the first year after agricultural abandonment, the subsequent rate of change is much slower and fungal biomass may increase only gradually over many decades before the fungal:bacterial biomass ratio starts to more closely resemble that of a natural ecosystem (van der Wal et al., 2006). Following the cessation of tillage

and fertiliser application the fungal community remains limited by other soil chemical properties, such as low C:N and low OM content which can take hundreds of years to return to levels seen in natural ecosystems (Knops and Tilman, 2000). The nature and rate of change in the soil fungal:bacterial biomass ratio following extensification of management has implications for nutrient cycling. Evidence suggests the rates of nutrient cycling (van der Heijden et al., 2008) and N losses via leaching and gaseous emissions (de Vries et al., 2011) are greater in bacterial dominated soils compared to soils with a high fungal biomass.

2.3.2 The potential effect of extensification on the soil biota

Sudden increases in populations of some trophic groups or species in soils could occur following land use change, such as the cessation of intensive agriculture. This could significantly alter nutrient cycling processes, for example increasing the availability of labile N (N that is readily transformed or used by micro-organisms) (Stutter et al., 2009).

The food web interactions that exist between soil animals and microbial communities are important in controlling nutrient cycling (Bradford et al., 2007). Many studies have shown that an increase in the population of consumers feeding on soil microbes can result in a greater release of nutrients into the soil system, thus stimulating nutrient cycles (Moore et al., 2003). Increased activity among certain soil microbial groups, following the cessation of intensive agriculture, could increase nutrient loss through soil erosion activities, due to enhanced microbial enzymatic decomposition of organic material, which could reduce colloid binding to the soil matrix (Tisdale and Oades, 1982).

2.3.3 Implications of a changing plant community for nutrient cycling

The risk of nutrient loss from bare soil under continuous agriculture is well documented, with farmers advised to minimise the period of bare ground, for example by retaining stubble or planting a cover crop (DEFRA, 2009). During the establishment of semi-natural communities on former agricultural sites, a long period of reduced vegetation cover could increase nutrient loss from soils. Immediately after the removal of existing vegetation and prior to the re-establishment of a new vegetation community, there is potential for the rapid loss of nutrients from bare

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and/or disturbed soil (Vitousek and Reiners, 1975). The risk is particularly great if introduced plants fail to establish or land is left deliberately bare and / or harrowed to aid the establishment of the desired species.

Crop and grass species grown under intensive agriculture differ from the plants that are encouraged under agri-environment schemes in their response to nutrient availability. Plant traits, such as photosynthetic rate and response to nutrient supply, can effect ecosystem properties including the rates of biogeochemical cycling, which in turn affect ES provision through changes in C storage and water quality regulation (Lavorel and Grigulis 2012).

Crop plant species and productive pasture grasses usually have morphological and physiological adaptations that maximise nutrient uptake. Plants adapted to low nutrient environments generally have a low nutrient uptake capacity, particularly for immobile nutrients such as P, for which uptake is highly dependent on the establishment of plant-microbe interactions (Aerts and Chapin, 1999). In response to high internal nutrient status, the specific rate of uptake of P (Breeze and Hopper, 1987) and N (Glass et al., 2002; Siddiqi et al., 1990) can be down-regulated by plants. Hence plant species that have evolved to survive in low nutrient environments, with a slow growth rate and lower demand for nutrients (Hobbie, 1992) will have a lower capacity to remove soil nutrients remaining after agricultural extensification.

An imbalance between nutrient supply and plant uptake could lead to increased nutrient losses. Evidence from agricultural sites shows that in years when climate limits plant growth, annual N losses in runoff are greatest, as slow plant growth reduces the rate at which N released from OM mineralisation is taken up from the soil (Vagstad et al., 1997). Similarly where shading or management such as clipping have reduced plant growth it has been found to increase soil inorganic N concentrations through decreased N uptake, as well as increasing net mineralisation due to decrease supply of plant derived C for immobilisation (Cheng et al., 2011). Where seed addition or removal of competitors (Smith et al., 2000) encourages the establishment of plant species adapted to low nutrient environments with lower productivity than crops (Walker et al., 2004), there is the potential for increased soil

nutrient losses similar to those that occur in years when crop growth rate is reduced by poor climate or management.

2.3.4 The importance of plant-microbe interactions and implications for nutrient loss following management change

Plant species composition has been shown to have a significant effect on soil microbial communities due to plant species specific effects on litter composition, root deposits and root exudates (Eisenhauer et al., 2010). Plants adapted to low nutrient environments tend to have lower relative growth rates, and produce litter of a lower quality (low % N and higher content of phenolics, lignin and structural carbohydrates), compared to plants from nutrient rich environments (Wardle et al., 2004). Therefore, after the cessation of intensive agricultural activities, it is expected that the plant derived material added to the soil would be less readily decomposed by soil microorganisms due to the higher content of recalcitrant compounds (Wardle et al., 2004). These changes may help limit harmful nutrient losses, since it has been shown that the addition of material with a high C:N ratio can result in a decreased $\text{N}_2\text{O} : \text{N}_2$ ratio due to increased N_2O reduction (Baggs et al., 2000).

Different types of C compound can interact with oxygen availability to regulate N_2O emissions from soil slurry (Morley and Baggs 2010). Nitrous oxide emissions were found to be lower following glucose and mannitol additions than following addition of butyrate or glutamic acid but the differences were only observed at higher initial oxygen concentrations in the incubation headspace. These findings show the potential for changes in plant exudates, and hence substrate supply, to alter microbially driven nutrient losses.

Agricultural ecosystems have been described as 'leaky' due to high quality litter inputs and a bacterial dominated microbial community causing rapid nutrient cycling and losses (Wardle et al., 2004). Frequent crop removal and high input of nutrients from external sources during intensive agriculture result in a decoupling of plant and soil microbial interactions (Haag and Kaupenjohann, 2001). It has long been considered that mature established ecosystems are, in contrast, less 'leaky' (Haines, 1977; Woodwell, 1974), due to slower, more efficient nutrient cycling (Wardle et al., 2004) and complex interactions between the plant and microbial community ensuring

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nutrients are well conserved (Richardson et al., 2009). These plant-microbe interactions include the release of nitrification inhibitors by plants in later successional stages (Lodhi, 1982) and plant associations with arbuscular mycorrhizal fungi, which can increase nutrient take-up by plant hosts. Hence nutrient losses via leaching and denitrification from established semi-natural, unfertilised grasslands have been regarded as insignificant, provided there is not excessive grazing (Woodmansee, 1978). However the legacy of intensive management practices on soil nutrients (discussed in section 2.1) may prevent the plant and microbial communities characteristic of nutrient poor environments from establishing, thus resulting in continued higher rates of nutrient loss. Furthermore it has been shown that the local adaptations of both plant and arbuscular mycorrhizal fungi to soil nutrient content are important in regulating formation and function of their associations (Johnson et al., 2010). Thus, where appropriate locally adapted plant propagules and associated fungi are not present for colonisation of former intensively managed agricultural land, it may take many years for systems with efficient nutrient cycling and low nutrient losses to establish.

Management that encourages diverse above ground plant communities does not always produce corresponding changes in the soil microbe community (Smith, R.S. et al., 2003). Changes in below ground food web structure have been found to lag behind changes in the plant community (Holtkamp et al., 2008). Following conversion from arable use to grassland at an experimental site in central Europe, Eisenhauer et al. (2010) found that microbial communities took 2- 4 years to respond to changes in plant species composition. The initial stages in the microbial community succession consisted of a transient community, shown to have lower C use efficiency than the more stable community that established later. During the transition period, after the cessation of intensive agricultural management ecosystem, nutrient cycling might be expected to be less efficient, with plant and microbial uptake being less well coupled to nutrient supply thus increasing the likelihood of potentially harmful nutrient losses. It is still not understood fully whether different functional groups within the soil community respond separately to plant succession, or whether the entire food web undergoes an integrated response (Maharning et al., 2009). A non-integrated response could lead to the decoupling of interactions

between trophic levels within the soil biota and potentially result in an even more 'leaky' system (Odum, 1985).

2.4 The legacy of intensive agriculture on soil physical properties and the significance for nutrient cycling

Reduced infiltration in compacted soils can result in more water leaving the site as surface runoff or in water only penetrating the tilled surface soils and not the compacted subsoil (Shafiq et al., 1994). This has been shown to increase the rate of soil erosion, and hence the loss of P associated with colloids and particulates (Addiscott and Thomas, 2000). Overall N losses to groundwater and the atmosphere are generally greater in compacted soil compared to un-compacted soil (Lipiec et al., 1995).

More compacted soils have lower availability of aerated pores; this has been shown to greatly increase N_2O emissions via denitrification (Bhandral et al., 2007). Nitrous oxide emissions from cut grassland sites have been found to increase exponentially as water-filled pore space (WFPS) increases from 50 to 90% (Smith et al., 1998b). The close relationship between N_2O emissions and soil WFPS has been attributed to an increased number of anaerobic zones in more saturated soil, where incomplete reduction of NO_3^- to N_2O can occur, although, when WFPS exceeds 90%, N_2O production was found to decrease due to further reduction of N_2O to N_2 (Smith et al., 1998b). Soil water content and BD are therefore important controls on the rate of nutrient loss in solution and through gaseous N_2O emissions.

The use of heavy machinery under intensive agricultural practices can lead to soil compaction and an increase in BD (Ball et al., 1999; Hamza and Anderson, 2005). Tillage can also break down soil macro-aggregates leading to a weak soil structure that is vulnerable to compaction (Hamza and Anderson, 2005). Although tillage loosens the surface soil, the use of heavy machinery may result in the formation of a hard compacted subsoil layer (Shafiq et al., 1994), particularly when machinery is used on wet soils. Soil compaction and the resulting decreased soil pore space cause a reduction in infiltration rate and soil moisture storage, such that soils become more rapidly saturated (Osunbitan et al., 2005). Once compacted soils may take many years to recover. Even when attempts are made to break up compacted soil with

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tillage or by encouraging earthworm activity, the soil structure remains weakened with fewer stable aggregates (Horn et al, 1995).

In agricultural land restored to native tall grass prairies in Nebraska, USA, soil BD decreased with increasing time since abandonment during the 12 year study. The gradual decrease over time following agricultural abandonment was attributed to increased inputs of labile C compounds in root exudates which bind together soil macro-aggregates (Baer et al., 2002), resulting in larger soil pore spaces. However, BD values were on average 21% higher 12 years after restoration than at non-cultivated sites (Baer et al., 2002). Therefore, rates of nutrient loss through gaseous emissions and leaching, may remain higher for years after land use change at sites formerly subjected to heavy machinery traffic.

Through its effects on soil structure, tillage can also alter heat energy flux through the soil profile (Johnson and Lowrey, 1985; Lipiec and Hatano, 2003) and therefore soil temperature. Temperature is an important variable affecting soil nutrient cycling and potential losses; for example, the rates of both denitrification (Smith et al., 1998b) and nitrification (Malhi and McGill, 1982) increase with increasing soil temperature. The activity of enzymes responsible for the break-down of organic soil N compounds is also positively correlated with soil temperature (Hallett et al., 2013). On sunny days compacted top soils tend to heat up and cool down more slowly and have smaller temperature fluctuations and lower spatial temperature variability than un-compacted top soil (Lipiec et al., 1991), whilst compacted soils tend to be warmer at depth. The differences between compacted and un-compacted soils were found to be greater at higher soil water contents (Lipiec and Hatano, 2003). Hence, whilst the waterlogging of compacted soil might encourage increased denitrification and loss of nutrients in leachate and runoff, this effect may be partially offset by reduced peak soil surface temperatures. The likely combined effect of any change in soil temperature and / or WFPS on nutrient cycling would partly depend on climate, as to whether temperature or soil water content is more limiting to nutrient loss processes at a given location. As already discussed, the impacts of intensive agriculture on soil structure may last for many years following extensification, leading to the maintenance of relatively high rates of nutrient loss.

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The nature of the surface vegetation, particularly its insulating effect and albedo, can also impact on soil temperatures (Lipiec et al., 1991; Song, 1999). Insulation from surface vegetation and litter, as found in low or no till ecosystems, has been found to reduce heat loss from soil overnight, resulting in greater average soil temperature (Franzluebbers et al., 1995). As grassland plants establish, total vegetation cover at extensively managed sites should increase, with a dense sward throughout the year. This is in contrast to cropped fields, where bare ground or stubble may be present for much of the year, and intensively grazed grasslands, where a shorter sward will be maintained. The presence of a long sward could result in higher average soil temperatures and potentially increased nitrification and denitrification rates. Local climate and soil texture will play a role in determining how the rates of nutrient loss via gaseous emissions and runoff are affected by soil temperature and moisture changes in response to change in the vegetation cover.

2.5 The efficacy of attempts at soil nutrient reduction following intensive production

Given the evidence of potentially high levels of nutrients remaining in soils after cessation of intensive agriculture, attempts have been made to reduce nutrient content at some sites entering agri-environment schemes by removing excess nutrients in plant uptake, with the primary aim of aiding the establishment of a diverse plant community. The main technique used is cropping with conventional crop species without fertiliser additions to encourage nutrient depletion prior to sowing of species with lower nutrient requirements (Walker et al., 2004). Nitrogen and potassium (K) may sometimes be added to ensure that their availability does not limit plant demand for P from the extractable soil pool (Tallowin et al., 2002). Nutrient removal by plant uptake from soil has been shown to vary with crop species, and is not always significant (McCrea et al., 2001). Nevertheless, the removal of plant available nutrients from soil by cropping and harvesting may be sufficient to allow the establishment and subsequent maintenance of SRG (McCrea et al., 2001). However, less plant-available nutrient forms may remain in the soil at high concentrations, thus there is still the potential for nutrient loss through gradual mobilisation of less plant available compounds. Hence, even at sites where measures

have been taken to reduce available soil nutrients, there is still a risk of substantial nutrient loss and a failure to achieve the aim of establishing a more species-rich sward.

2.6 Environmental and Economic costs of nutrient loss from agricultural soils after land use change

Much of the evidence presented so far in this review indicates a potential for sustained nutrient losses from soil after the cessation of intensive agricultural activity. In this section the costs of these potential losses to the farmer and the environment are assessed.

2.6.1 Environmental costs of nutrient loss in leachate and runoff

Nutrient losses from agriculture to water-bodies are globally significant. Modelled estimates of nutrient export from rivers (Seitzinger et al., 2005) showed that globally 62% of dissolved inorganic N export was from non-point anthropogenic sources, mostly agricultural sources, and was derived from fertiliser applications and biological N fixation. Where N and P are limiting nutrients, increased concentrations in water bodies can result in eutrophication (Søndergaard and Jeppesen, 2007). Even in the case of P, where losses tend to be small compared to the total soil concentration, persistent chronic losses can have detrimental effects on the ecology and amenity value of fresh water and coastal ecosystems (Dungait et al., 2012, Kleinman et al., 2011). There is also a threat to human health posed by toxic algal blooms and high concentrations of nitrates in drinking water (Townsend et al., 2003, Sutton et al., 2012). Nutrients leached in forms that are readily available for biotic uptake, such as NO_3^- , may have relatively large, immediate effects on the aquatic system close to the source. Less labile nutrients, such as DON and P, can accumulate over time in groundwater (Holman et al., 2008) and in sediment deposited in lakes and coastal regions, where they contribute to eutrophication (Seitzinger and Sanders, 1997) and can be a source of N_2O emission (Kroeze and Seitzinger, 1998).

2.6.2 Environmental costs of N_2O and NO emission from soil

Nitrogen lost from soil in gaseous form as N_2O , influences atmospheric chemistry and contribute to global climate change. Nitrous oxide is a potent greenhouse gas with a global warming potential 298 times greater than CO_2 for a 100-year time

horizon (IPCC, 2007) it also contributes to stratospheric ozone depletion (Vitousek et al., 1997). The effect of management extensification schemes on N₂O emissions from soil are not well characterised. Perturbations to soil N cycling, or increases in soil N content following cessation of intensive agriculture, could result in significant N₂O production and emissions. Nitric oxide (NO) is another gas with harmful environmental consequences that can be produced in the soil by nitrification, denitrification and chemo-denitrification (Yamulki et al., 1997); it contributes to the formation of tropospheric ozone, which impacts on both human health and plant growth (Vitousek et al., 1997). As with N₂O, NO production is dependent on soil temperature and soil water content, with N₂O production by denitrification favoured over NO when soil moisture content is high (Bollmann and Conrad, 1998; Götter and Conrad, 1999; Smith, K.A. et al., 2003). Thus, under conditions which favour NO production, the negative environmental impacts are in part balanced by a co-occurring decrease in N₂O production, each gas having different associated costs.

2.6.3 Economic costs of nutrient losses from agriculture

Any loss of nutrients from agricultural soils represents a cost in terms of clean-up and restoration of aquatic ecosystems, drinking water treatment and loss of amenity value of waterways. The annual costs of freshwater eutrophication are estimated to be \$2.2 billion in the USA (Dodds et al., 2009) and \$105-160 million in England and Wales (Pretty et al., 2003). Removal There are also indirect costs to the farmer and society including: paying for fertiliser which is not ultimately of benefit to crop production; the energy used for fertiliser production and application (Haag and Kaupenjohann, 2001); and loss of P, a non-renewable resource (Oelkers and Valsami-Jones, 2008).

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In order to address the hypothesis set out in Chapter 1, the characteristics of pairs of field plots were compared. Each plot pair consisted of:

1. An extensively managed SRG plot, which had undergone conversion from intensive agricultural management. As set out in section 1.6, the SRG plots were no longer receiving fertiliser, and had been sown with a grassland seed mix following removal of the original crop / pasture. Some management was still undertaken including in some cases light grazing, where permitted under the requirements of the agri-environment scheme, and cutting.
2. An intensively managed (IM) plot representing the same management that had been applied at the SRG plot prior to conversion. Management at IM plots included fertiliser application, tillage and liming.

Field plots were located at sites on working farms in South East Scotland and at Rothamsted Research North Wyke in South West England (here after referred to as North Wyke), with contrasting management histories, soils and climates. This chapter describes the field site locations, soil type and land use. It then describes the methods and protocols used for sample collection in the field and subsequent analysis. Where the methods used differ between the Scottish farms and North Wyke (NW) this is clearly stated and each method fully described.

3.1 Locating Scottish field plots

3.1.1 Selection of field sites on Scottish farms

The study was focussed on working farms in the Borders region of South East Scotland. This area was chosen due to its proximity to the University of Edinburgh (Figure 3.1) to minimise travel time and cost. The location also offered suitable sites for research, with recently established SRG sites ranging from 3 to nine years old. Under the SRDP, regional priority BOR08 for the Borders region is to achieve “a halt in the loss of biodiversity and reverse previous losses through targeted action” (The Scottish Government, 2011a). One of the packages proposed within this priority is the creation of SRG. Species rich grassland creation is also suggested as a package

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to help reduce diffuse pollution, another regional priority for the Borders region (Scottish Government, 2011b). The Scottish field sites were selected from SRGs which had been created under the SRDP.

Potential field sites were identified with the aid of farm advisers at the Scottish Agricultural College (SAC), who provided contact details for 8 farmers who had received their help in applying for SRDP funding for the creation of SRGs. Farmers were initially contacted by letter to introduce the aims of the project and the types of data that would be collected. Farmers were then contacted by phone to establish whether, having read the letter, they would be willing to take part in the study and to obtain more details on the size and age of the SRG fields, and to ascertain whether suitable IM fields existed that were adjacent to and represented the former management of the SRG. Finally, a visual inspection was undertaken to establish that there were areas within the SRG that matched parts of the intended IM sites in slope and aspect, soil samples were examined to determine the broad soil texture class using hand texturing to it was similar at both the IM and SRG sites. Discussions were also carried out with farmers to determine suitable plot size and location with IM and SRG sites, to ensure they were happy with the planned location of field equipment and sampling points. A total of four site pairs were selected from three farms. The farm from which two site pairs were selected was a large farm formed from a collection of what were once smaller farms, the distance, aspect, soil type and management of the two selected sites were considered different enough and the sites far enough away from each other so as to ensure the between site differences between them were similar to those between the other pairs.

The working nature of the farms meant that only a single plot could be located in each IM field so as to minimise disruption, as such 4 sites pairs were selected and served as the 4 replicates. This number was decided as the maximum that could be managed, given the time and resources available and sufficient to provide preliminary data towards answering the hypotheses laid out. Where possible each round of data collection was conducted at all sites pairs within a three day period so as to minimise between site differences due to sampling date, where this was not possible due to timing of management activities preventing access to certain sites it is

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made clear. It was noted that detailed policy recommendations might require a much larger dataset from a greater number of UK sites, and that this work would provide only initial data to indicate the most important areas for future research and provide an indication of where policy efforts should be focused.

3.1.2 Location of plots within field sites

One square plot 11 m x 11 m was set up at each IM and SRG plot. The plot location was first identified at the IM site for each site pair in consultation with farmers, so as to be near to field access points and to minimise disturbance of the crop by vehicles and researchers, whilst ensuring that the plot was located at least 20 m from the edge of the cultivated area of the field to minimise the impact of any edge effects. Once the location for the IM plot had been agreed, a suitable region within the paired SRG site was located, which matched the slope and aspect of the IM plot. Within this region, coordinates for the centre of the plot were selected using random numbers.

3.2 Site characteristics and management of Scottish sites

Each Scottish site is identified by the letter S followed by a number, which refers to the age in years since establishment of the SRG, which is different for each site. As such the SRG plots form a chronosequence of increasing age. The data collected from the sites were analysed to determine any trends with increasing age of the SRG (Section 4.3) however the site pairs differed from one another in many respects other than age of the SRG, including soil type, location, slope and management history, therefore the lack of significant trends with increasing age of the SRG plots for the majority of soil variables was unsurprising. Farmers were promised anonymity at the start of the project, so only a rough indication of location can be given. Farmers were asked for details of the historical and on-going management at each site, but it was not always possible to get all the desired information. All the information provided by farmers is presented in this chapter.

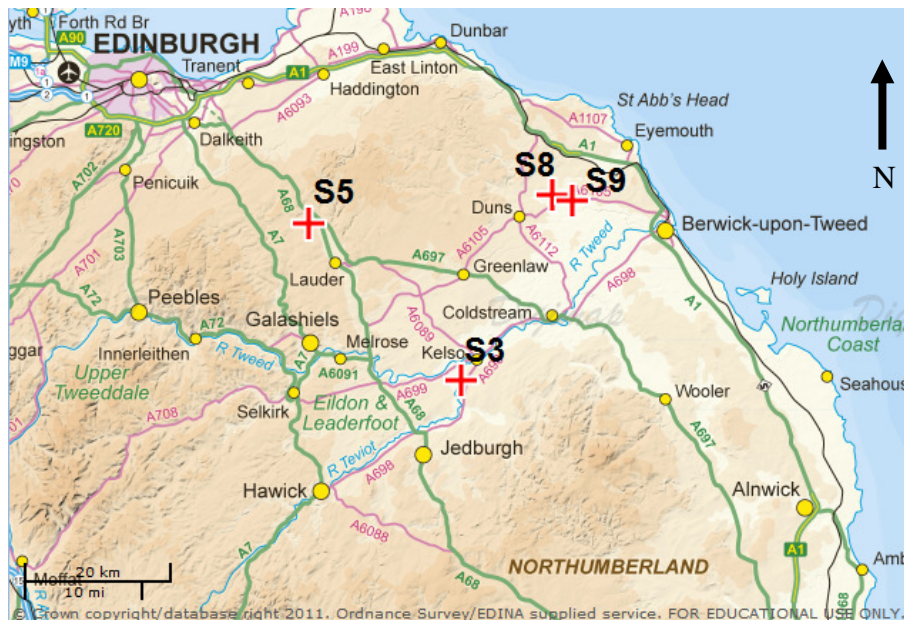


Figure 3.1 Location of Scottish field sites, map sourced from Ordnance Survey (2011a)

3.2.1 Site S3

Site S3 is located approximately 80 km south-east of Edinburgh, on a large arable farm. Sampling at the site commenced in March 2010, the 3rd year since the SRG was entered into the SRDP, and was completed by July 2012. Prior to 2008, both the IM and SRG plots were under the same arable rotation. In spring 2008 the SRG entered the SRDP, with the sowing of a commercial seed mix (Table 3.1). Once entered into the SRDP, the SRG no longer received fertiliser and was cut for hay in March and at the end of August every year and was not grazed. The IM plot remained under arable rotation growing cereals including oats (*Avena sativa*), barley *Hordeum vulgare*), wheat (*Triticum spp.*) and rye (*Secale cereal*), with rye being grown in 2010. The field was rented out for potato growing in 2011; this did not form part of the standard rotation that had been used at the site over previous years. The renting of the field meant that soil gas and water samplers (Section 3.0 and 3.11) could not be deployed in 2011 but soil samples could still be collected (Section 3.6).

The IM and SRG plots were originally part of the same field but were separated when the SRG was entered into the SRDP. At one edge of the SRG there was a stream with steep banks, however the area of the SRG adjacent to the IM field was

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very similar in slope, aspect and drainage so this area was selected for the location of the SRG plot (Figure 3.2). The soils under each plot were of a sandy loam texture (methods and results of soil texture analysis in section 3.6.2 and appendix A) and contained virtually no stones (rock fragments > 2 mm diameter) as these had been previously mechanically removed to aid cultivation from both the SRG and IM fields.

Table 3.1 Seed mix sown at site S3 SRG

Species Name	Common Name	% by weight
<i>Phleum pratense</i>	Timothy grass	32
<i>Dactylis glomerata</i>	Cock's foot	16
<i>Festuca rubra</i>	Strong creeping red fescue	8
<i>Agrostis castellana</i>	Highland bent	2
<i>Festuca pratensis</i>	Meadow fescue	37
<i>Poa pratensis</i>	Smooth meadow grass	5



Figure 3.2 Site S3 SRG (front/right) and S3 IM (back/left) with dividing fence, which was erected when the SRG was entered into the SRDP.

3.2.2 Site S5

Site S5 is located approximately 30 km south-east of Edinburgh on a small farm (approximately eight hectares). Sampling at the site commenced in March 2010, which was the 5th year since the SRG was entered into the SRDP and ended in January 2011 when the SRG came out of the SRDP scheme. In 2001 the farm converted from entirely arable growing spring barley (*Hordeum vulgare*) to intensive grassland which was cut for hay and occasionally lightly grazed. In 2005, following discussions with SAC advisers, fertiliser application to the field intended for conversion to SRG was stopped to encourage utilisation of plant available nutrients by the existing grass sward. In spring 2006, this field was then converted to SRG under the SRDP, the existing grass sward was ploughed up and a commercially available seed mix sown (Table 3.2). Once entered into the SRDP the SRG no longer received fertiliser applications and was cut for hay in the middle of September and lightly grazed for 3-4 weeks, from the middle of October to early November, every year. The IM plot remained under intensive grassland management, with no grazing, twice yearly fertiliser application and cutting for hay in July and September.

The IM and SRG plots were in two separate fields, separated by a drainage ditch and fence (Figure 3.3). The IM (S5IM) and SRG (S5SRG) plots were located in almost flat areas with silty loam textured soils, which contained some stones (> 2mm) (approx.. 4 per 500g soil collected). The methods and results of soil texture analysis are presented in section 3.6.2 and appendix A.

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Table 3.2 Seed mix sown at site S5 SRG

Species Name	Common Name	Origin	% by weight
<i>Agrostis tenuis</i>	Common bent	cultivated	13
<i>Alopecurus pratensis</i>	Meadow foxtail	cultivated	4
<i>Cynosurus cristatus</i>	Crested dogs tail	cultivated	13
<i>Festuca rubra ssp. litoralis</i>	Slender creeping red fescue	cultivated	34
<i>Poa pratensis</i>	Smooth meadow grass	cultivated	21
<i>Achillea millefolium</i>	Yarrow	Fife	1
<i>Centaurea nigra</i>	Common knapweed	Fife	2.5
<i>Galium verum</i>	Lady's bedstraw	Fife	0.7
<i>Hypochaeris radicata</i>	Cat's ear	Fife	0.2
<i>Lathyrus pratensis</i>	Meadow vetchling	Fife	0.2
<i>Leontodon autumnalis</i>	Autumn hawkbit	Fife	0.1
<i>Leucanthemum vulgare</i>	Ox-eye daisy	Fife	2.3
<i>Plantago lanceolata</i>	Ribwort plantain	Fife	2.5
<i>Prunella vulgaris</i>	selfheal	Fife	2
<i>Ranunculus acris</i>	Meadow buttercup	Fife	2
<i>Rhinanthus minor</i>	Yellow rattle	Inverness-shire	0.5
<i>Rumex acetosa</i>	Common sorrel	Fife	0.5
<i>Vicia cracca</i>	Tufted vetch	Fife	0.5



Figure 3.3 S5 SRG (foreground) and S5 IM (background) with dividing fence and ditch

3.2.3 Site S8

Site S8 is located approximately 80 km east-south-east of Edinburgh, on a large mixed farm. Sampling at the site began in March 2010, the 8th year since the SRG was entered into the SRDP and was completed by July 2012. There is no physical divide between the SRG and IM areas at the site. Prior to 2003, both were under the same arable rotation producing cereal crops including oats, wheat, barley and rye. In spring 2003 the SRG entered the SRDP, with the sowing of a commercial seed mix (Table 3.3). Once entered into the SRDP the SRG no longer received fertiliser, it was cut in March and at the end of August every year and was not grazed. The IM area remained under arable rotation, with rye being grown in 2010 and wheat in 2011.

One edge of the SRG is more steeply sloping than the rest of the field. The region of the SRG closer to the IM area was very similar in slope and aspect to the IM area, so this region was selected for the location of the SRG plot (Figure 3.4). The soil texture in each plot was a silty loam and the soils were very stony. The methods and results of soil texture analysis are presented in section 3.6.1 and appendix A.

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Table 3.3 Seed mix sown in SRG at sites S8 and S9

Species Name	Common Name	% by weight
<i>Cynosurus cristatus</i>	Crested dog's tail	25.5
<i>Festuca ovina</i>	Sheep's fescue	34
<i>Poa pratensis</i>	Smooth meadow grass	17
<i>Agrostis capillaris</i>	Common bent	8.5
<i>Papaver rhoeas</i>	Corn poppy	2.4
<i>Ranunculus acris</i>	Meadow buttercup	1.3
<i>Centaurea nigra</i>	Common knapweed	1.3
<i>Lotus corniculatus</i>	Common bird's foot trefoil	1.3
<i>Leucanthemum vulgare</i>	Oxeye daisy	1.2
<i>Prunella vulgaris</i>	Self-heal	1.8
<i>Achillea millefolium</i>	Yarrow	0.6
<i>Rumex acetosa</i>	Common sorrel	1.3
<i>Rhinanthus minor</i>	Yellow rattle	3.75



Figure 3.4 Site S8 SRG (right) and S8 IM (left)

3.2.4 Site S9

Site S9 is located on the same large mixed farm as site S8. Sampling at the site began in April 2010, the 9th year since the SRG site was entered into the SRDP, and was completed by July 2012. Prior to 2002 the SRG and IM fields were under the same arable rotation growing cereal crops. In spring 2002 the SRG entered the SRDP, with the sowing of the same commercial seed mix as at S8SRG (Table 3.3). Once entered into the SRDP, the SRG no longer received fertiliser; it was cut in March and lightly grazed by cattle at the end of August every year. The IM field remained under arable rotation, with rye being grown in 2010 and wheat in 2011.



Figure 3.5 Site S9 SRG (left) and S9 IM (right)

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The SRG and IM plots were in adjacent fields, with very similar slope, aspect and drainage (Figure 3.5). The soil texture under both plots was a silty loam and the soils were very stony. The methods and results of soil texture analysis are presented in in section 3.6.1 and appendix A.

3.3 North Wyke plots

Rothamsted Research North Wyke (NW) is a grassland research farm in South West England to the north of Dartmoor (Figure 3.6). It provides carefully controlled and replicated experimental plots. The SRG plots used for this research formed part of a pre-established experimental site on former intensive grassland. Sampling at the plots began in March 2010 and continued until August 2012



Figure 3.6 Location of NW field sites, map sourced from Ordnance Survey (2011b)

3.3.1 Management history at North Wyke

Prior to 1980, the entire NW farm was managed as a commercial farm; with cereal crops, such as winter oats (*Avena sativa*) grown. In 1981 the site was left under stubble for 12 months before the Institute of Grassland and Environmental Research (IGER) began managing the farm in October 1981. After this, the entire farm was managed as a research facility, predominantly as intensive grassland with clover rich swards and fertiliser additions, although crops were grown intermittently. It should be noted that the management of the site for intensive arable production prior to 1981 could itself still be having a legacy effect, as such although the land use change

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under investigation at North Wyke is for the purpose of this study considered as intensive grassland to extensive grassland, the results may differ from other sites which have never been under arable production. The extent of this legacy of historical management could only be determined by a larger scale study where additional sites could be investigated. The SRG was converted from IM in 2008, as part of an extensification experiment (Wide Scale Enhancement of Biodiversity). No further fertiliser additions were made and the site was divided into plots. A range of treatment combinations were applied to the plots and each treatment combination was replicated four times. The design of the plot layouts was determined using an adapted Latin square design (see appendix C for the layout of the NW SRG plots).

3.3.2 Selection of North Wyke plots

The SRG plots selected for sampling for this research were the four replicate plots of a single treatment, chosen to most closely resemble that encouraged in Scotland under the SRDP. The selected plots all measured 25 m x 35 m, during the 2008 conversion to SRG they were ploughed to remove the existing grass sward and then sown with a seed mix of grass, legumes and forbs (Table 3.4) to resemble the species rich mixes specified in the SRDP. The selected plots were not grazed, as only minimal grazing is encouraged under the SRDP. Instead, the selected plots were those that were cut under a typical management regime for the farms in the region, with the sward cut and removed at the beginning of June and end of August each year.

For comparison with the four NW SRG plots, four IM plots were sited in a field, approximately 1.5 km from the established SRG; the IM plots were also part of the research farm but remaining under intensive grassland management. The IM field received fertiliser additions at least twice yearly and was at times grazed and in some years cut for hay.

The locations of the four IM plots were selected so that each SRG plot was paired with an IM plot that matched it for slope and soil type, according to the NW soils classification. The Soils under plots 1-3 are categorized as Halstow Clays, and under plot 4 the soil is a Hallsworth Clay (Harrod and Hogan, 2008).

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Sampling was carried out in plots 1-4 between March 2010 and September 2011 plots 1-3 in 2012, due to disturbance at plot IM4 in order to construct a drainage, sampling in 2012 was not possible.

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Table 3.4 Seed mix sown in NW SRG plots

Species	Common Name
<i>Alopecurus pratensis</i>	Meadow foxtail
<i>Dactylis glomerata</i>	Cock's foot
<i>Festuca pratensis</i>	Meadow fescue
<i>Lolium perenne</i>	Perrenial ryegrass
<i>Phleum pratense</i>	Timothy grass
<i>Achillea millefolium</i>	Yarrow
<i>Centaurea nigra</i>	Common knapweed
<i>Cichorium intybus</i>	Common chicory
<i>Leucanthemum vulgare</i>	Oxeye daisy
<i>Lotus corniculatus</i>	Birdsfoot trefoil
<i>Medicago lupulina</i>	Black medic
<i>Melilotus officinalis</i>	Yellow melilot
<i>Onobrychis viciifolia</i>	Sainfoin
<i>Rumex acetosa</i>	Common Sorrel
<i>Sabguisorba minor</i>	Salad burnet
<i>Trifolium hybridum</i>	Alsike clover
<i>Trifolium pratense</i>	Red clover
<i>Trifolium repens</i>	White clover

3.4 Summary and comparison of North Wyke and Scottish sites

The Scottish sites provide data from working farms that have entered the SRDP. However, the commercial nature of the farms limited the area that could be covered by sampling plots, preventing multiple replicate plots from being established within a site, although multiple farms could be used to provide four site pairs. NW provided a single SRG and IM field, at which multiple replicate IM and SRG plots could be established due to the farm's status as a designated research platform (Rothamsted Research, 2013). A further contrast is that the Scottish sites had a predominantly arable management history, with the exception of S3, which had recently been converted to grassland prior to entrance into the SRDP, whilst NW had been managed as an intensive grassland system for over 20 years prior to conversion of the SRG site. Another key contrast between the Scottish and NW sites is the climate in the two regions of the UK, the most notable difference being the mean annual rainfall (Table 3.5). A summary of the key features of all site pairs is provided in Table 3.6.

Table 3.5 30-year (1971-2000) mean air temperature and rainfall for field site regions (Met Office, 2011)

Site(s)	Mean annual temperature (°C)	Mean winter temperature (°C)	Mean summer temperature (°C)	Mean annual rainfall (mm)
Scottish	6-9	1.5-3.5	13.5-15.5	400-900
NW	7-9.5	2.5-5	12-15	1400-1800

3.5 Sampling soil chemical and physical properties

Physical and chemical soil properties were determined on a number of occasions at the Scottish and NW sites, as shown in Table 3.7. The soil properties for analysis were selected as being those most likely to impact on ES provision potential at the sites, as well as being likely to be affected by intensive agriculture (see chapter 2 and appendix F).

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At the Scottish sites, available N and P were analysed in both spring and summer 2010/2011 to record changes in more labile nutrient pools over the growing season. The March / April soil samples were collected following crop sowing in the IM plots at sites S3, S8 and S9 and prior to the first fertiliser application to all IM plots. The July soil samples were taken at least two weeks after the previous fertiliser application to ensure all previously applied fertiliser had entered the soil or been taken up by plants. Soil pH was also measured twice in each year to detect any short term changes due to plant root secretions and break down of organic matter. Total soil N, P and C concentration were determined only in the spring of each year, as the total soil nutrient pool includes the more recalcitrant mineral compounds, whose concentrations in soil change much more gradually than those of the more labile available compounds (Section 2.2). Similarly BD was determined only in the spring of each year as this has been shown to be slow to change (Section 2.4), hence is relatively stable over shorter periods of time. Soil moisture was determined for use in calculations of nutrient concentrations and WFPS (Section 3.6). Soil samples were collected from two depth ranges, 0-10 cm, which contained the greatest density of plant roots, and 30-40 cm, below which the C horizon was reached; soil texture and structure visibly contrasted to the soil above. In the summer samples were only collected from the 0-10 cm depth range, which is the layer most likely to show intra-annual changes due to the nutrient cycling between the soil, soil biota and plant roots (Section 2.3).

Analysis of soil samples from NW was carried out in order to provide data for input to and comparison with the output from the UK-DNDC model, as well as to compare intra-annual variation in available nutrient and WFPS between paired plots, as both are key factors influencing N₂O fluxes (Chapter 2).

3.5.1 Summary of frequencies and timings of soil analyses at North Wyke and Scottish sites

Table 3.7 provides a summary of the occurrence of each type of soil analysis on both Scottish and NW soils. In addition soil moisture was determined for every gas sampling occasion at the Scottish sites in 2010 and 2011 and on each gas sampling occasion in 2012 at NW. Soil sample collection in March / April at the Scottish sites

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was conducted prior to the first fertiliser application of the year and July sampling was at least two weeks post any fertiliser application to the IM plots

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Table 3.6 Summary of the characteristics of each site pair

Site Pair	Year since establishment of SRG (in 2010)	Period of data collection	Number of plots per site	Management prior to SRG conversion (Both IM and SRG)	SRG management post conversion	IM management post conversion	Soil Type
S5	5	March 2010-Jan 2011	1	Spring barley until 2001 then intensive grassland	Cut and lightly grazed	Intensive grassland (cut)	Silty loam
S3	3	March 2010-July 2012	1	Arable rotation	Cut	Rye (2010), potatoes (2011)	Sandy loam
S8	8	March 2010-July 2012	1	Arable rotation	Cut	Rye (2010), wheat (2011)	Silty loam
S9	9	March 2010-July 2012	1	Arable rotation	Lightly grazed	Rye (2010), wheat (2011)	Silty loam
NW	3	March 2010-July 2012	4-2010 & 2011 3-2012	Intensive grassland (1981 onwards)	Cut	Intensive grassland (cut and grazed)	Clay

Table 3.7 Summary of incidence of each soil chemical and physical analysis including depth and site from which samples were taken. Analyses conducted as described in section 3.5 and including available N (Av. N), available P (Av. P), total N (Tot N), total P (Tot P), total C (Tot C), pH, organic matter (OM), bulk density (BD), and soil moisture (SM). 'Y' indicates samples were collected

Date	Depth (cm)	Site	Av. N	Av. P	Tot. N	Tot. P	Tot C	pH	OM	BD	SM	Texture
Mar- 10	0-10	S3, S5,	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	30-40	S8, S9	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Jul- 10	0-10	S3, S5,	Y	Y				Y			Y	
	30-40	S8, S9										
Sep- 10	0-10	NW			Y		Y				Y	
	30-40											
Jan- 11	0-10	NW	Y	Y				Y			Y	
	30-40											
Apr- 11	0-10	S3, S8,	Y	Y	Y	Y	Y	Y	Y	Y	Y	
	30-40	S9	Y	Y	Y	Y	Y	Y	Y	Y	Y	
Jul- 11	0-10	S3,S8,	Y	Y				Y	Y		Y	
	30-40	S9										
May -12	0-10	NW							Y	Y		
	30-40	plots 1-3										
Mar- June -12	0-10	NW	Y									Y
	30-40	plots 1-3										

3.6 Sampling and analysis of soil chemical and physical properties at the Scottish sites

Soil analysis methods are detailed below. Unless stated otherwise the frequency and timings of sampling is provided in section 3.5.1. Each sample collected from the field was approximately 500 g in weight to ensure there was sufficient for all analyses. Soil samples were stored in a cool box after collection in the field, then in a freezer immediately on return from the field to minimise continued mineralisation of organic matter and chemical transformation of N and P compounds once sampled. Samples were allowed to defrost in a fridge before preparation and analysis. Before analysis fresh soil was passed through a 2 mm sieve, and collected in a tray. Gloves were worn to do this and the tray and sieve were washed with de-ionised water between each sample to prevent cross-contamination of N and P compounds between samples. Sieved soil was stored in the fridge if necessary for up to three days.

3.6.1 Soil sampling strategy at Scottish sites

At the Scottish sites, five soil samples were collected per plot on each occasion to represent spatial heterogeneity within each plot, whilst ensuring analysis costs were not prohibitively large. Unless otherwise stated, samples were collected using a 5 cm diameter soil corer. To ensure that the samples were representative of the whole plot and not collected from ground disturbed during insertion of gas sampling equipment (Section 0), a variation on the commonly used five point “W” arrangement was used. Soil samples were broadly taken from a “squashed X” design (Figure 3.7), although often the exact position of sampling was adjusted to avoid stones when inserting the corer.

3.6.1 Soil texture

Particle size analysis was carried out on soils from the Scottish sites at the start of the research in March 2010 to verify that the soil texture was similar at paired IM and SRG plots. Soil was sampled from five points (Section 3.6.1) over a 0-40 cm depth range for each plot. The soil from each plot was bulked and passed through a 2 mm sieve. Sieved soil was then air dried at room temperature overnight. The samples were placed overnight in an ultrasound bath in 2:1 mass ratio of 4% sodium hexametaphosphate : soil. A Beckman Coulter LS230 particle size analyser at The

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University of Edinburgh was used to determine the % by volume of particles of different sizes. Soil particles were defined according to their diameter as sand (0.06 - 2 mm), silt (0.002 - 0.06 mm) or clay (<0.002 mm), and the textural class of soil was defined based on the relative proportions of the three particle types according to the system of the British Standards Institution (British Standards Institution, 2006). Soils at all paired sites were found to be of the same textural class (results in appendix A), which allowed further comparisons of dependent variables to be carried out.

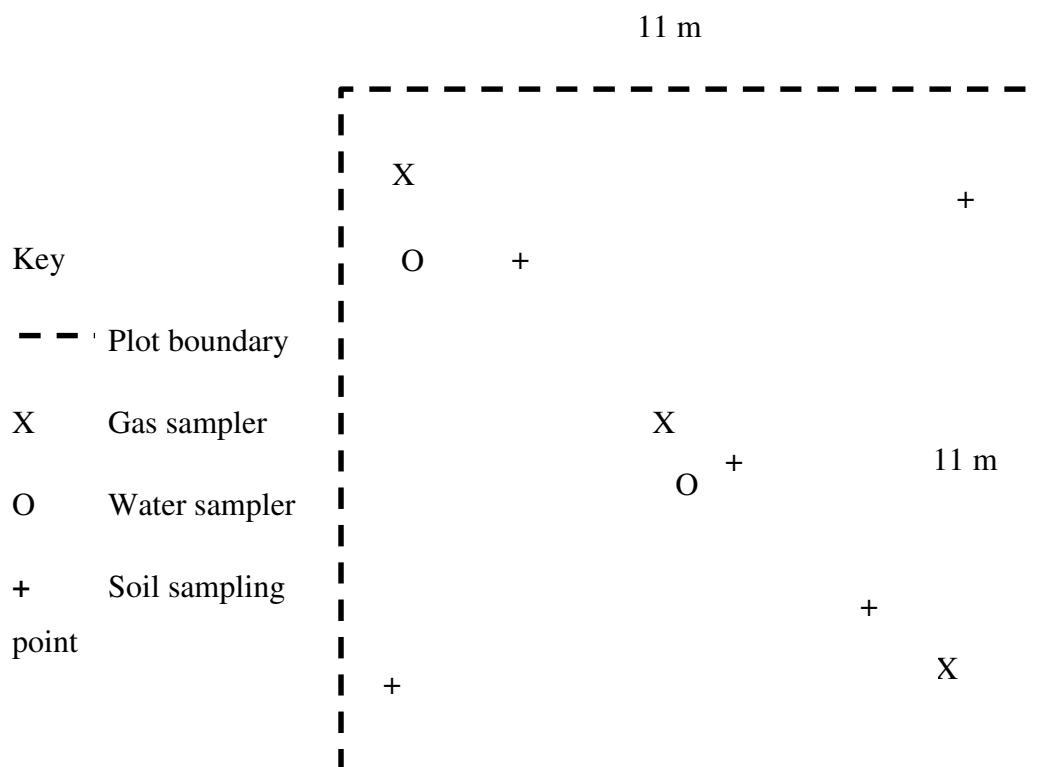


Figure 3.7 Approximate location of soil sampling points within Scottish plots

3.6.2 Soil Bulk Density

Soil BD of the surface soil at the Scottish sites was determined using steel cores (56 mm diameter and 40 mm depth; Eijelkamp, Giesbeek the Netherlands). Vegetation and roots were removed from the soil surface and the core was pushed into the ground until the edge of the rim was flush with the soil surface, taking care not to compact the soil (Gifford and Roderick, 2003). The cores were labelled and returned

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to the lab intact. The soil was removed from the cores and passed through a 2 mm sieve, the sieved soil was weighed then oven dried (Section 3.6.5). The volume of stones and roots removed from each Scottish soil sample was determined by measuring the water displacement in a 100 ml cylinder. Equation 1 was used to calculate the soil BD (Don et al., 2007). Five soil cores were taken from each plot.

$$BD (g\ cm^{-3}) = \frac{\text{Mass dry soil (g)}}{(\text{Volume of core} - \text{Volume of stones})(cm^{-3})} \quad (1)$$

3.6.3 Soil pH

In the laboratory, 10 (± 0.1) g of a soil sample was weighed into a plastic cup and 25 ml of deionised water was added. The mixture was stirred for a few seconds and then left for 20 minutes to reach equilibrium. The pH was then measured with a Fisher Brand Hydrus 300 pH probe (Fisher Scientific, Loughborough UK) calibrated using pH 4 and 7 buffer solutions after every 10 samples.

3.6.4 Soil OM concentration

Soil OM concentration was determined by measuring loss on ignition (Davies, 1974). Approximately 2 g of oven dried soil (Section 3.6.5) was weighed into a crucible of known weight. The crucible and soil were placed in a furnace at 450 °C for 10 hours, (Donkin, 1991) the ashed soil was then allowed to cool in a desiccator. The crucible and soil were then re-weighed. Equation 2 was used to determine the % OM content of the soil.

$$\% OM = \frac{(\text{Mass of dried soil} - \text{Mass of ashed soil})}{\text{Mass of ashed soil}} \times 100 \quad (2)$$

3.6.5 Soil moisture content and water filled pore space

Gravimetric soil moisture content was determined for all soil samples to allow conversion of analyte concentrations, determined for fresh samples, to concentration in dry soil. Soil moisture content was also determined on gas sampling occasions, as it has a strong influence on soil N₂O fluxes. Approximately 20 g of fresh soil was removed from each sieved sample prior to chemical analysis and weighed in a foil container of known mass. The soil was dried in an oven at 105 °C for 24 hours. The sample was removed from the drying oven and allowed to cool for 1 hour in a

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desiccator and then re-weighed. The sample was then returned to the oven and dried for a further five hours and then cooled and re-weighed as before. This procedure was repeated until there was no further decrease in mass between successive weighings. All masses were measured to the nearest mg. Equation 3 was used to calculate the soil moisture content.

$$\% \text{ Soil moisture} = \frac{(\text{Mass fresh soil} - \text{Mass oven dry soil})}{\text{Mass oven dry soil}} \times 100 \quad (3)$$

Soil moisture content was used along with soil BD (Section 3.6.2) and organic matter content (Section 3.6.4) to calculate WFPS using Equations 4 – 6. A particle density of 2.65 g cm^{-3} and 1.3 g cm^{-3} was assumed for soil mineral and organic particles respectively (Ilstedt et al., 2000).

$$\text{Particle density} = (\text{OMC} \times 1.3) + ((1 - \text{OMC}) \times 2.65) \quad (4)$$

$$\text{Soil porosity} = 1 - \left(\frac{\text{Bulk density}}{\text{Particle density}} \right) \quad (5)$$

$$\% \text{ WFPS} = \frac{\text{Soil water content (g g}^{-1}\text{)}}{\text{Soil porosity}} \times \frac{\text{Bulk density}}{\text{Density of water}} \times 100 \quad (6)$$

Where:

$$\text{density of water} = 1 \text{ (g cm}^{-3}\text{)}$$

3.6.6 Available N

Available N, defined as the sum of ammoniacal N ($\text{NH}_4^+\text{-N}$), nitrate N ($\text{NO}_3^-\text{-N}$) and nitrite N ($\text{NO}_2^-\text{-N}$) concentrations, was determined on fresh soil samples. Five $5 (\pm 0.02) \text{ g}$ of fresh soil was weighed into a 200 ml glass bottle and 100 ml of 6% potassium chloride (KCl) according to a commonly use protocol (Dorich and Nelson, 1982; Pansu and Gautheyrou, 2006 p777). The 200 ml glass bottles were placed on an orbital shaker for an hour at $150 \text{ revolutions min}^{-1}$. Once removed from the shaker the bottles were left to stand for 10 minutes to allow the suspension to settle. Twenty ml of the solution was then filtered through Whatman No. 42 filter paper (Whatman plc Maidstone, UK) into a sample vial.

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Two reagent blanks (bottles containing KCl solution only and no soil) were also prepared for each run and processed in an identical manner as samples and the results subtracted from the samples to control for any contamination during the procedure. Because time did not allow all the samples in the run to be replicated, five samples were randomly selected for replicate analysis to ensure reliability of the method. In all cases results for the two replicated samples differed by less than 5%.

A Bran & Luebbe Auto Analyser III running methods G-102-93 and G-109-94 (as described by Keeney and Nelson (1982)) was used to measure NH_4^+ and $\text{NO}_3^-/\text{NO}_2^-$ concentrations respectively. For NH_4^+ the sample was reacted with salicylate and dichloroisocyanuric acid, with a nitroprusside catalyst to give a blue compound, whose absorbance was measured at a wavelength of 660 nm. The combined concentration of $\text{NO}_3^-/\text{NO}_2^-$ was measured by first reducing NO_3^- to NO_2^- , through reaction with hydrazine in alkaline solution with a copper catalyst. The NO_2^- then reacted with sulphanilamide and naphthylethylenediamine dihydrochloride (NEDD) to form a pink compound whose absorbance was measured at a wavelength of 550 nm. The absorbance of sample solutions was compared to the absorbance of standards of known concentrations of NH_4^+ and NO_3^- . Equations 7 and 8 were used to convert the concentration of each analyte in the sample solutions (mg l^{-1}) to concentrations in dry soil (mg kg^{-1}). Concentrations of NH_4^+ and NO_3^- were then converted to $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-/\text{NO}_2^-\text{-N}$, although the total concentration of both oxidised N forms (NO_3^- and NO_2^-) was measured it was assumed for this purpose that NO_2^- concentrations in most acid soils are negligible relative to NO_3^- (Shen et al., 2003).

$$\text{Mass dry soil (g)} = \frac{\text{Mass fresh soil (g), i.e. 5 g}}{\left(\frac{\% \text{ Soil moisture content}}{100}\right) + 1} \quad (7)$$

$$\begin{aligned} \text{conc. of analyte } x \text{ (mg kg}^{-1} \text{ dry soil)} = \\ \frac{\left(\text{Sample conc.} - \text{Mean blank conc. (mg l}^{-1})\right) \times (100 + (\text{Mass fresh soil} - \text{Mass dry soil (g)}))}{\text{Mass dry soil (g)}} \end{aligned} \quad (8)$$

3.6.7 Available P

Available P, defined as acetic acid extractable P, was determined for a separate subsample of soil, using the same method of extraction as for available N (Section 3.6.6) but replacing the KCl solution with 100 ml of 2.5% acetic acid (Edwards and Hollis, 1982; MISR/SAC, 1985). The concentration of phosphate (PO_4^{3-}) in the solution was measured using a Bran & Luebbe Auto Analyser III running phosphate method G-103-93, in which the PO_4^{3-} was reacted with molybdate and ascorbic acid, in the presence of potassium antimonyl tartrate catalyst, to form a blue compound (John, 1970) whose absorbance was measured at 660 nm. Equation 8 was used to calculate the concentration of P in dry soil.

3.6.8 Total P

The Kjeldahl method was used to determine total P in the soil samples (Taylor, 2000). Two replicate digests were carried out for each sample and with each batch of nine replicated samples 2 reagent blanks were included, such that digestions were carried out in batches of 20. A mass of 0.5 (± 0.005) g of oven dry soil was added to a 300 ml test tube with six Kjeldahl catalyst tablets containing copper sulphate (Fisher Scientific, Loughborough UK) and 20 ml of 95% sulphuric acid H_2SO_4 . Tubes were then placed in a fume cupboard in a Buchi K-437 digestion system (Buchi UK Ltd, Oldham UK) and heated for 30 mins at 250 °C then for 90 mins at 350 °C. Once cool, de-ionised water was carefully added to the digests and the solutions were filtered through Whatman No. 42 filter paper (Whatman plc, Maidstone UK) into a 250 ml volumetric flask and made up to volume with deionised water. The flask was sealed with a stopper and hand shaken for a few seconds then left for 10 hours to reach equilibrium. Sixty ml of the solution was poured into a vial and stored in a refrigerator prior to analysis by a Bran and Luebbe autoanalyser III, using the same method as for available P. Equation 9 was then used to convert the concentration of P in the digest to the equivalent mass of total P in the soil sample (mg kg^{-1} dry soil). All digests were analysed in duplicate

$$P \text{ conc. (mg kg}^{-1} \text{ dry soil)} = 500 \times (\text{Sample P conc. (mg l}^{-1}) - \text{Blank P conc. (mg l}^{-1})) \quad (9)$$

3.6.9 Total soil C and N

Approximately 15 mg of oven dried (Section 3.6.5), ground (pestle and mortar) soil was weighed out into a foil capsule and a Carlo Erba NA 1500 analyser was used to obtain the % elemental mass of N and C in the sample. Each sample was analysed in duplicate.

3.7 Sampling soil chemical and physical properties at North Wyke

Soil analysis at NW comprised of that required to generate data to support the UK-DNDC model (Section 5.6) or PLFA analysis (Section 3.8). Two hundred g samples were taken from five points in each plot arranged in a “W” pattern (standard Rothamsted procedure). Soil moisture and organic matter content were measured on site at NW, using the same methods as described in section 3.6. The method for measuring soil BD differed at NW slightly to that used in Scotland. Soil samples were collected using a soil corer measuring 2.5 cm in diameter and 7.5 cm in depth. The high clay content of the NW soils meant it was difficult to pass them through a sieve so instead any large stones or roots (> 2 mm in diameter) were removed following visual inspection. For other chemical and physical analysis NW soils were either frozen immediately on collection then couriered in cool bags with ice packs to Edinburgh (journey time approx. 10 hours), or posted first class as fresh samples if being analysed for available N. On reaching Edinburgh the soils were immediately stored in a fridge, prior to analyses in the Edinburgh labs using the procedures already described (Section 3.6).

3.8 PLFA analysis

The soil microbial community was characterised for the NW plots by analysis of phospholipid fatty acids (PLFAs). The cell membranes of living microbes contain phospholipids which are composed of a phosphate moiety bound to a glycerol moiety which is bonded via ester linkages to two fatty acid moieties (Figure 3.8).

Phospholipids are rapidly degraded on cell death, and certain fatty acid groups are only found in the cell membranes specific taxonomic groups. Therefore analysis of soil PLFA content enables the size and broad structure of the living soil micro-biota to be determined (Frostegård and Bååth, 1993).

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Soil samples collected in 2012 on 5 March (pre-fertiliser application to the IM plots), 11 Mar (post fertiliser application), 19 April and 17 May were selected for analysis to enable any temporal changes in microbial community composition to be identified, additionally these dates represented a range in soil mineral N and N₂O flux measurements (Sections 5.4 and 5.5), which would enable any correlation between variations in the soil microbial community composition and other soil parameters to be identified, for each of the three SRG and IM plots.

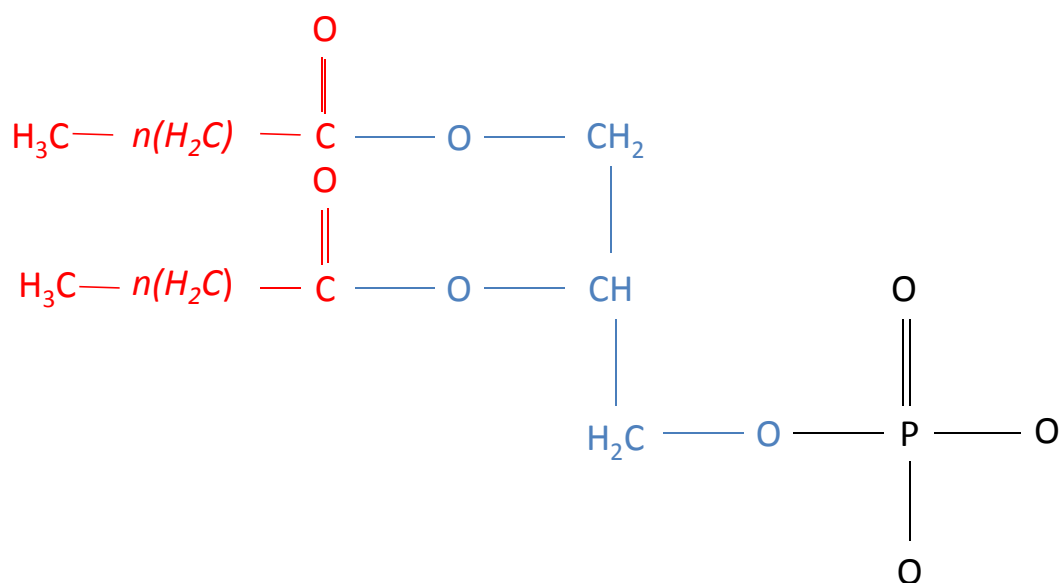


Figure 3.8 The structure of a phospholipid showing the phosphate group (black), glycerol backbone (blue) and fatty acid moieties.

For each plot, soil for PLFA analysis was taken from 0-10 cm depth from the five sampling points (Section 3.6.1) with a metal trowel that was cleaned and sterilised with alcohol to minimise the risk of contamination. In addition, extreme care was taken to avoid touching the soil samples at all stages of the sampling and analysis procedures. The soils from each plot were bulked and wrapped in aluminium foil and stored at -20 °C at NW.

Prior to analysis frozen samples were freeze-dried using an Edwards Pirani 501 Super Modulyo freeze dryer (Edwards Ltd, Crawley UK). The freeze-dried samples from each of the five sampling points in each plot were ground and homogenized in a

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room fitted with a dust extraction and ventilation system using a Retsch, RM 200 Mortar Grinder (Retsch, Haan Germany), which was thoroughly cleaned with, Microl Sol³⁺ (Anachem, Luton UK) anti-microbial decontaminant 1:10 dilution, acetone and methanol between each sample.

Extraction of PLFAs was carried out using a modified Bligh Dyer extraction method (Bligh and Dyer, 1959) according to Crossman et al. (2004), although methylation was conducted using acid methylation in place of methanolic boron trifluoride (Sasser, 1990). All measuring cylinders and bottles were cleaned prior to use by washing with laboratory grade cleaner (Decon, Hove UK) and then rinsing twice with HPLC (high performance liquid chromatography) grade methanol then twice with HPLC grade hexane. All other glassware, including Pasteur pipettes, was wrapped in aluminium foil and heated a muffle furnace at 410 °C for four hours to remove any organic matter contamination prior to use. The lids of all sample vials were lined with foil to reduce the risk of sample contamination from plastic derived lipids. PLFA extractions were carried out on batches of 6 soil samples. Within each batch a reagent blank was included to check no sample contamination was occurring. The following section gives detail on the method of PLFA extraction used.

3.8.1 Preparation of solvents

Buffered water:

One litre of Millipore water (Millipore, Billerica MA USA) was added to a separating funnel along with 25 ml HPLC grade DCM (dichloromethane), the funnel was stoppered then fully inverted several times. The stopper was removed to allow gases to escape and the aqueous and organic layers were allowed to separate, and the lower organic phase was run off as waste. This washing stage was repeated twice more, then 500 ml of the DCM extracted water was measured out and transferred to a separating funnel for buffering. First 3.4 g of potassium dihydrogen phosphate (KH₂PO₄) was weighed out onto a piece of foil and added to the separating funnel to give a 0.05 M solution. The funnel was stoppered then inverted and shaken several times to mix. Then sodium hydroxide (NaOH) was added gradually to the funnel, which was inverted and shaken after each addition until the pH had reached 7.2. The buffered water was then washed with DCM three times as described above

Bligh Dyer Solvent (BDS):

The BDS was made by mixing 4 parts buffered water (prepared as described above) with five parts chloroform (CHCl_3) and 10 parts methanol (MeOH). The BDS was stored in a tinted glass bottle for no longer than one month.

3.8.2 Total lipid extraction

Five grams (± 0.001 g) freeze dried soil was weighed along with 10 ml BDS into a 28 ml sample vial. The vial was vortexed for 10 seconds, then ultrasonicated for 15 minutes, before being centrifuged at 2000 rpm for 5 minutes. Using a Pasteur pipette, the supernatant was transferred to a second 28 ml vial, a further 2 ml BDS was added to the original sample vial and the extraction was repeated twice more. Five ml of CHCl_3 and 5 ml buffered water were then added to the supernatant, vortexed for 10 seconds and centrifuged at 2000 rpm for 5 minutes to separate the organic and inorganic phases. The organic phase (lower layer) was transferred to a round bottomed flask, the phase separation of the supernatant was repeated twice more with 3 ml each of CHCl_3 and buffered water. The organic phase was rotary evaporated using a Buchi rotary evaporator under vacuum (Buchi UK Ltd, Oldham UK) at 40 °C. The residue was re-dissolved in 3 x 1 ml of 2:1 (v/v) DCM:iso-propanol (iPrOH) and then dried by passing through a column of anhydrous sodium sulphate (Na_2SO_4 , activated at 105 °C) produced in a Pasteur pipette plugged with extracted cotton wool, the column was conditioned with 4 ml 2:1 (v/v) DCM:iPrOH. The solvents were then evaporated from the solution under nitrogen gas (N_2) at 40 °C. At this point vials could be sealed with PTFE (polytetrafluoroethylene) tape and stored in a fridge or desiccator overnight

3.8.3 Fractionation to isolate polar (phospholipid) fraction

The sample was re-dissolved in 0.5 ml CHCl_3 , a silica gel (particle size 60A activated at 125 °C) column was produced in a glass pipette, the column was conditioned with 2 x 1 ml MeOH, followed by 2 x 1 ml CHCl_3 before the sample was added. Following sample addition, the neutral lipids were eluted with five times 1 ml CHCl_3 , the glycolipids were eluted with 5 x 1 ml acetone and finally the phospholipids were eluted with 5 x 1 ml MeOH and collected in a test tube. The solvent from the phospholipid fraction was evaporated under N_2 at 40 °C

3.8.4 Saponification of phospholipids

Saponification of the phospholipids was carried out to cleave the fatty acids from the glycerol and phosphate moieties (Figure 3.8) by breaking the ester bonds. Methanolic NaOH was produced by dissolving 4 g NaOH in 10 ml DCM extracted water, 90 ml MeOH was added to the NaOH (aq), the solution was sonicated for 10 minutes. One millilitre of methanolic NaOH was added to the sample in each test-tube. A glass syringe was rinsed 6 x with hexane, then used to add 20 μ l of 0.1 mg ml⁻¹ of hexane nonadecane (C19 *n*-alkane) to the sample. The sample was then heated at 80 °C for 1 hour in a vial sealed with PTFE tape. One molar hydrochloric acid (HCL) was produced by adding 6 ml concentrated HCL to 48 ml DCM extracted water. Once cool, approximately 3 ml of the 1 M HCL was added to the sample until pH paper indicated it had reached pH 1 - 2. Two millilitre of diethyl ether was added to the sample which was then vortexed, the diethyl ether (top) layer was extracted into another test tube, extraction with diethyl ether was repeated twice more. The solvent was evaporated under N₂ at 40 °C.

3.8.5 Acid methylation of phospholipids

Methylation was carried out in order to generate fatty acid methyl esters (FAMES), which could then be separated via gas chromatography and identified based on their mass spectra. A stock of MeOH was dried by shaking with excess anhydrous Na₂SO₄ that had been furnace at 550 °C. The MeOH was acidified by adding 1 ml H₂SO₄ to 49 ml MeOH. Two millilitre of acidified dry MeOH was added to each sample; sample tubes were then sealed with PTFE tape and heated at 70 °C for 1 hour. Once cool, 2 ml DCM extracted water was added to the sample, the organic phase was then extracted in 3 x 1 ml hexane, the sample was vortexed before each 1 ml extraction to ensure mixing of the phases. The solvent from the extract was evaporated under N₂ at 40 °C, then dried on a Na₂SO₄ column, produced as above but this time the column was conditioned and the vial was rinsed with three times 1 ml hexane. The hexane was evaporated from the sample under N₂ at 40 °C. The sample was stored under N₂ in a freezer prior to analysis via gas chromatography – mass spectrometry (GC/MS).

3.8.6 Gas Chromatography Mass Spectrometry (GC/MS)

GC/MS an Agilent 6890N gas chromatograph interfaced to an Agilent 5973 Network mass spectrometer (Agilent Technologies UK Ltd., Wokingham, UK) was used to identify and quantify the PLFAs. The sample was re-dissolved in 500 μ l hexane (50 drops with a Pasteur pipette) and 1 μ l injected into the GC inlet where it was vaporized and swept onto a chromatographic column by the carrier gas (helium; 9.33 psi, 8.8 ml min⁻¹). The injection syringe was rinsed twice in 2 separate hexane washes between samples. The sample flowed through the column and the compounds were separated by virtue of their relative interaction with the coating of the column (stationary phase; J&W DB225; 30 mm x 0.25 mm i.d x 0.25 μ m phase thickness) and the carrier gas (mobile phase). Oven temperature was set at 80 °C for 1 minute, the temperature then increased at 15 °C min⁻¹ up to 180 °C, then at 3 °C min⁻¹ up to 215 °C, this temperature was then held for 3 minutes, the total run time was 23.3 minutes. The latter part of the column passes through a heated transfer line and ends at the entrance to ion source where compounds eluting from the column are converted to ions. The next component is a quadrapole mass analyser (filter), which separates the positively charged ions according to their mass. After the ions are separated they enter a detector the output from which is amplified to boost the signal. MSD ChemStation D.01.02.16 software (Agilent Technologies UK Ltd., Wokingham UK) was used to integrate the peaks and to display the mass spectra for identification of the FAMES see section 5.3.1.

The notation used to describe individual FAMES throughout this study gives the C chain length followed by a colon then the number of double bonds and the position (number of C atoms) of the double bond from the methyl end (ω) of the molecule, the letters c and t indicate the cis / trans isomerism respectively of the double bond. The prefixes *i* and *a* identify *iso* and *anteiso* branched C chains, 10-Me is used where a methyl group is located on the 10th C atom from the carboxyl end of a molecule and *cy* indicates a cyclopropane fatty acid.

The phospholipid FAMES were analysed with selected ion monitoring (SIM) by following ions with a mass to charge ratio of (*m/z*) of 74 and 199. As an exception, the latter ion had *m/z* 268 for 16:1 acids, *m/z* 250 for *cy*-17:0, *m/z* 298 for *i*-18:0 and

18:0, m/z 294 for 18:2 ω 6, m/z for 18:1, m/z 312 for 10-Me-18:0 and 19:0, m/z 278 for cy-19 and m/z 326 for 20:0. Soil gas flux

3.9 Soil gas flux

Using the static chamber technique, air samples were collected and analysed for N₂O at all sites. At NW the GC also analysed gas samples for CO₂ concentration. The chamber bases were installed in the plots and, where possible, left in situ between samplings to minimise soil disturbance. Although it was necessary to move the Scottish chambers for ploughing of the IM fields and grazing and cutting of the SRG, the design of the chambers used at NW (described in section 3.9.2 below), meant they could remain in situ. Air samples were collected between 10:00 and 14:00 hrs, as it has been shown that sampling at these times provides a good representation of the average daily flux given the diurnally fluctuating nature of soil N₂O emissions (Yamulki et al., 1995; Cardenas et al., 2010). During sampling the chambers were closed for 40 minutes and a gas sample taken at the start (T0) and end of closure (T40) to determine the change in gas concentration in the chamber headspace over the closure period. Previous work has shown 40 minutes to be the optimum closure time at agricultural sites in the UK, with gas accumulation within the headspace occurring at a constant rate over this time period (Smith and Dobbie, 2001; Cardenas et al., 2010), closure for a longer time would risk a decrease in rate of flux between the soil and chamber headspace due to a decreasing concentration difference between the two. All gas samples were analysed within 14 days of collection; this time period has been shown to give minimal change in gas concentration for the type of vial used (Hansen et al., 1993). Soil surface (0 - 2 cm depth) temperature in the shade was also measured by inserting a digital thermometer (Thermosense Ltd., Bourne End UK) into the soil at a near horizontal angle adjacent to each chamber (Shepherd et al. 1991). Equation 10 was used to determine the N flux in (g N ha⁻¹ day⁻¹), derived from the measured N₂O concentrations.

N flux from soil =

$$(N_2O \text{ conc. at } T40 - N_2O \text{ conc. at } T0) \times \frac{28}{22.4} \times \frac{(273 + \text{soil surface temp}(^{\circ}C))}{273} \times \\ \text{chamber height (m)} \times \frac{10000}{1000} \times \frac{60}{\text{closure time}} \times 24 \quad (10)$$

3.9.1 Chamber design and sampling procedure at Scottish sites

Three chambers were placed in each Scottish plot (Section 3.6.1), with approximately 7.5 m between the chambers, although in some cases it was necessary to offset chambers slightly from the planned location due to stony soil inhibiting insertion. The mean N_2O flux from the three chambers was taken to estimate the overall flux from each plot. The bases for the chambers used at the Scottish sites were made from thick circular plastic piping. They protruded 0.4 m above the ground, to allow space for vegetation when closed, and were inserted to a depth of 10 cm into the soil (Figure 3.9) after using a metal cutting shoe to create a ring within the soil in which to insert the base of the chamber. Once inserted the soil was pushed against the chamber edge to ensure a tight seal. On each sampling occasion the metal chamber lids were fixed to the bases with four pipe clips (Figure 3.10). A ring of draft excluder was fixed to the lids at the point of contact with the chamber rim to create a seal and a 5 ml syringe with a three way tap valve was inserted through a hole in the centre of the lid and sealed with a circular washer to form a sampling port. Prior to each sampling, the seals on the lid and around the port were checked to ensure they were not worn and replaced if necessary. After insertion the mean height of the chamber headspace (required for equation 10) was calculated by taking the mean of 15 measurements of the distance from the soil surface to the base of a plane held flush against the chamber rim.



Figure 3.9 Gas sampling chamber base used at Scottish sites



Figure 3.10 Gas sampling chamber with lid used at Scottish sites

Samples from all six chambers in paired plots were collected almost simultaneously with the three chambers in each plot being closed successively, with a 2 minute gap between each, there was a 2 minute gap before closure of the chambers in the second plots commenced, again at 2 minute intervals. Gas samples were collected in 20 ml Chromacol vials with a chlorobutyl septum (Chromacol, Welwyn Garden City, UK), which were evacuated using a hand pump to a pressure of -70 kPa. The vials were then filled with air from the chamber, the chamber port valve was immediately closed again and the vial was re-evacuated and refilled with chamber air. This was repeated four times, with the 4th sample of headspace air being retained for analysis. The samples were analysed at the University of Edinburgh using an Agilent model 6890 gas GC (Agilent Technologies UK Ltd., Edinburgh, UK) fitted with a 1.8 m Haysep Q column and electron capture detector (ECD). A Hewlett Packard automatic sampler, modified with a SGE 2.5 ml gas-tight syringe to sample 50 20 ml gas filled vials, was used. The sampler was controlled with PeakSimple Chromatography Data System software (Chromtech) and a methane-argon carrier gas (BOC, UK) was used. Nitrous oxide concentrations of the samples were determined using a calibration curve produced from samples of known concentration run through the GC, a new calibration curve was produced after every 10 samples to account for instrumental drift. The calibration curve was generated from three samples of known concentration, including an atmospheric air sample collected in the field (0.31 ppm), and 2 standards of 1 ppm and 10 ppm (BOC, UK).

3.9.2 Chamber design and sampling procedure at North Wyke

Four chambers were emplaced in each NW plot with 5 m between each chamber (Figure 3.11). The mean flux from the four chambers was used to estimate the overall flux from each plot. The rectangular bases for the chambers used at the NW plots were metal rims that were flush with the soil surface. This ensured the chamber bases did not interfere with grass cutting or grazing and so could remain in place throughout the sampling. Some problems were encountered in the IM plots with cattle standing on and distorting the bases. When this occurred it was necessary to remove the bases and reshape them. A cutting shoe was used to cut a slot in the soil for the chamber bases and care was taken to ensure the chamber base was flush with the soil around the entire circumference. The chamber lids were plastic boxes that were painted white to reflect sunlight and minimise temperature increase inside the headspace during closure (Figure 3.12). A 5 mm thick layer of adhesive neoprene was fixed to chamber lids around the surface of contact with the bases to ensure a tight seal; they were attached to the bases with hooked bungee cords which were passed through holes drilled in the bases. The chamber ports were formed from rubber tubing passed through a sealed hole in the lids with a three way tap valve.

Key

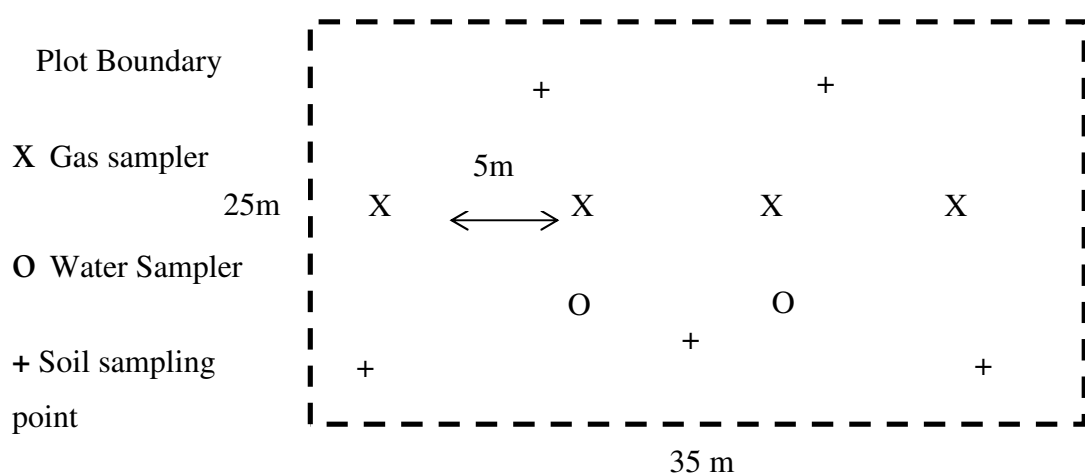


Figure 3.11 Location of static chambers, soil water samplers and soil sampling points within NW plots



Figure 3.12 Gas sampling chamber design used at NW

Due to the distance between the SRG and IM field, it was necessary to sample from all four plots in one field and then all the plots in the other field. Within each set of four plots, there were two minutes between closures of successive chambers, travel time between the two fields was 10 minutes. The order in which the two fields were sampled was determined randomly to limit directional bias in fluxes due to the difference in sampling time between the two fields and there was never more than two hours between sample collections at paired plots. Samples were collected in 20 ml Perkin Elmer vials with chloro-butyl septa (Perkin Elmer, Waltham MA USA). Prior to sampling the vials were evacuated using 21G hypodermic (microlance) needles (BD Franklin Lakes NJ. USA), attached to an electric wall mounted pump, which were inserted for five minutes through the vial septum to produce a vacuum of -1 bar. In the field, a 50 ml syringe was flushed three times with air from the chamber before a sample from the chamber port was injected into a pre-evacuated vial with a 21 G microlance needle. A 25 G microlance needle was also pierced through the septum to let out excess gas and ensure the sample in the vial was at atmospheric pressure. On the first two sampling occasions, the samples were analysed for N₂O concentration only using a Hewlett Packard 5890 series II manual injection GC. Peak areas for standards were used to produce a calibration curve for converting peak areas of samples to equivalent N₂O concentration as described above. Standards used were all BOC standards (BOC, UK) with concentrations ranging from 1-20 ppm, standards and were run every 10 samples to check for instrumental drift. After the first two sampling occasions, a Perkin Elmer Clarus 500

Chapter 3 Site description and methods

GC with a TurboMatrix 110 auto headspace sampler became available. The GC included an ECD set at 300 °C and a Perkin Elmer EliteQ PLOT megabore capillary (30m × 0.53 mm i.d.) column, operated at 35 °C with N₂ as the carrier gas. All subsequent samples were analysed using this system, for N₂O and CO₂ sample concentrations were calculated automatically by comparing sample peak area with those of known standards. Although not directly reported, the CO₂ fluxes from each chamber were calculated to verify that they were similar for all chambers in a plot and used as an indication that the chambers were not leaking.

3.9.3 Summary of gas sampling dates for Scottish and North Wyke sites

Due to low fluxes being measured on the first sampling occasions at all sites, it was decided that sampling should be more concentrated around times when N₂O and CH₄ emissions would likely be higher, such as immediately after rainfall and freeze thaw events (Smith and Dobbie 2001). In 2012 sampling at NW was most concentrated around the times of fertiliser addition at the IM plots, cutting of either set of plots and rainfall events.

3.9.4 The UK-DNDC model

The UK-DNDC model was used to simulate the fluxes from the NW sites in 2012 for comparison with field measurements. Detail on the inputs used for the model is given in section 5.6

3.10 Plant species identification and analysis

3.10.1 Identifying plant species present in Scottish SRG plots

The plant species present in the SRG plots at the Scottish sites were surveyed in July of 2010 and 2011. A 1 m x 1 m quadrat, subdivided into 0.1 m x 0.1 m segments, was placed at five points within each plot selected using random numbers. 1 m² quadrats are commonly used for sampling European grasslands (Pywell et al., 2007) and, given the diversity of the sward and the time and resources available, it was decided that this would be the optimum number and size of quadrats for sampling the plots (Rodwell, 2006). All plant species within each quadrat were identified by two surveyors (C. Horrocks and another). Dicotyledons (dicots) and monocotyledons (monocots) were identified with the help of keys from Rose (2006), and Hubbard

(1992) respectively. Where possible identification was made in the field, but where closer examination was required samples were returned to the lab and stored in a cool box in polythene bags with a few drops of water to keep them moist. An estimated value for percentage ground cover was agreed upon by the two surveyors for each species. A % cover was also estimated for bare ground where present. For analysis, the species were grouped according to their classification as grass / forb, and according to whether or not they were present in the seed mix. Ellenberger indicator values (Ellenberg et al., 1979), describing the realized ecological niche in terms of: soil N availability, light availability, soil pH and soil moisture, were obtained for each species. Ellenberg values were obtained from Hill et al. (1999).

3.10.2 Identifying plant species present in North Wyke plots

Plant species data were collected by NW staff from the SRG plots as part of the on-going wider experiment. Ten 1m x 1m quadrats were placed alternately on either side of a mid-line transect through each plot, with the distances along and perpendicular to the midline chosen randomly for all quadrats. On each side of the midline, alternate quadrats were surveyed either for vegetation cover or frequency, such that an estimate of cover was provided for all species in a total of five quadrats and the presence / absence of forb species was recorded for the other five quadrats in each plot. Plant species were also surveyed in the IM plots for comparison with the SRG plots; sampling was carried out by Claire Horrocks. Five 1 m x 1 m quadrats were randomly located within each plot and the % cover of identified plant species was estimated.

3.11 Soil water sampling

Prenart 'super quartz' soil water samplers (Prenart Equipment Aps. Frederiksberg Denmark) were installed in all plots in Scotland and NW for soil water sampling; however limited success was achieved with the method (described in detail in appendix B). At NW, the samplers only collected sufficient water for analysis during the late autumn and winter, when the soil water content was highest due to low evapo-transpiration and high rainfall. The requirement for high soil water content in order to obtain a sample, could explain the lack of soil water collection at the Scottish sites, where average rainfall is lower (Section 3.4) and soils are sandier and

Chapter 3 Site description and methods

more free draining compared to the soils at NW (Sections 3.2 and 3.3). Soil water samples were successfully collected from the NW plots in January and then again in February / March 2010. However, cattle that grazed the IM field during 2011 caused damage to some of the samplers. It was decided that due to the time and cost of replacing the NW samplers and analysing the samples, along with the limited success of obtaining water samples at the Scottish sites, soil water collection should be discontinued from 2011 onwards.

3.12 Data analysis

Data analysis was carried out using Microsoft excel unless otherwise stated. Specific detail of the analyses used for each set of results is given at the start of the results chapters. Where P values are provided a value of ≤ 0.05 is considered significant, unless otherwise stated. Prior to the use of a T-test, a check for normality of variance was carried out using an F-test for homogeneity of variances in Microsoft excel, where the F – statistic suggested the population variances were significantly different, a heteroscedastic t-test was carried out. A randomized block ANOVA design was used to analyse soil chemical data, with the data blocked according to the site pair (S3, S5, S8 and S9), to reduce the noise in the datasets due to site differences. Separate models were written for the spring (March / April) and summer (July) data. The spring data were analysed using a split plot design split two ways, first for soil depth (0-10 cm / 30-40 cm) then for the year (2010 / 2011). The summer soil samples were taken from 0-10 cm depth only, so the ANOVA design was split one way.

Chapter 4 Results and discussion of data from Scottish sites

4.1 Chapter structure

This chapter provides details of data analysis and presents all the data collected from the Scottish field sites, a description of the field sites and methods used is given in Chapter 3. Once all the results have been presented the findings are then discussed, in order to compare the potential for ES provision from the IM and SRG plots and determine the extent to which the hypotheses presented in Chapter 1 are supported.

The first data presented are climate data; daily rainfall and minimum / maximum temperature for the sampling period (2010 / 2011), are compared with the 30-year mean values for the region. Climate can have an important role in regulating soil physical conditions, including water content and temperature. Soil physical properties in-turn affect the potential for nutrient loss in leachate and runoff, as well as plant and microbial community structure and function. Consequently, climate can have an important regulatory effect on the rates of nutrient cycling and loss and hence the provision of a range of ESs.

Soil chemical properties, such as the availabilities of different nutrients, are key factors regulating rates of nutrient cycling and harmful losses. Soil chemistry influences the composition and functioning of plant and soil microbial communities. Detailed sampling of a range of soil chemical properties was undertaken at the Scottish sites. The data produced are presented and analysed to ascertain the extent to which they support hypotheses 1 and 2 (Section 1.7), and subsequently used to help explain the findings of the plant community analysis and N₂O flux measurements, and to compare the potential for provision of other ES' from the IM and SRG plots.

Following the results from soil chemical analysis the data from the plant species survey are presented. A key aim of establishing SRG is to provide enhanced biodiversity, the analysis of the data will assess the extent to which this is achieved and whether or not hypothesis 3 (Section 1.7) should be rejected.

The next data presented are the results from soil temperature and WFPS measurements, carried out alongside measurements of N₂O fluxes. Soil physical

properties, including soil temperature and WFPS are affected by the climate and vegetation and, along with the soil chemical properties, can be a major factor influencing the rates of microbial metabolism and nutrient loss including the N₂O flux from soil.

Finally the N₂O flux data are presented and the fluxes compared between paired IM and SRG plots to investigate field measurements support hypothesis 5. The regulation of greenhouse gas emissions is a key ES of interest to this study, and N₂O fluxes are strongly influenced by the ecosystem services presented throughout the chapter, hence these data are the last to be presented in this chapter.

4.2 Climate data.

In order to help interpret the N₂O fluxes measured at the Scottish sites, this section summarizes the monthly mean daily maximum temperature (MDmax) and mean daily minimum temperature (MDmin) and rainfall totals for South East Scotland for 2010 / 2011, alongside the 30-year mean from 1971-2000.

4.2.1 Obtaining climate data

The 30-year (1971-2000) MDmax and MDmin were obtained from the UK Met Office (Met Office 2012a), values from two Met Office stations are presented; Central Edinburgh, at 61m AMSL, (above mean sea level) is an urban environment, and Boulmer is a small village on the Northumberland coast at 23 m AMSL (Figure 4.1). These locations were chosen as the field sites were roughly equal distance between them. The 2010 / 2011 temperature data used were obtained from a private meteorological (met.) station in Greenlaw, a small town in the Scottish Borders at 200 m AMSL (Borders Weather, 2012). The station forms part of the Scottish Weather Network, the UK Weather Network and the Interactive Independent Climate Change project (IICCP) and the data collected are made publically available online.

Average rainfall totals can differ un-predictably across small distances, so comparison of 30-year mean rainfall in Edinburgh and Boulmer and 2010 / 2011 data from Greenlaw was considered inappropriate. It was decided that in order to fairly compare the rainfall in 2010 / 2011 with the 30-year mean, it was preferable to use the same dataset to calculate the 30-year mean and the 2010 / 2011 monthly totals.

Chapter 4 Results and discussion from Scottish sites

Data were obtained from the UK Met Office (Met Office 2012b). The data used were those published for the North East of England and consisted of the adjusted average rainfall totals, calculated from measurements taken at multiple rainfall stations from across the region. This dataset was chosen as the north east of England is the closest climatic region to the field sites.



Figure 4.1 Locations of met stations at Edinburgh, Greenlaw and Boulmer along with Scottish field sites.

4.2.2 Analysis of climate data

Analysis of climate data was carried out to compare the rainfall totals and daily minimum / maximum temperatures over the sampling period with the 30-year mean, to determine how typical the climate was across the sampling period. To account for the differences in altitude between the met. stations, the 30-year mean MDmax and MDmin temperatures from Edinburgh and Boulmer were adjusted, assuming an environmental lapse rate of 0.65°C for every 100 m increase in altitude (O'Hare et al., 2005), the 2010 / 2011 Greenlaw MDmin and MDmax values were left unadjusted. The difference between the 30-year mean and the 2010 / 2011 MDmax and MDmin was calculated by subtracting the 2010 / 2011 data, from the mean of the two altitude adjusted 30-year means from Edinburgh and Boulmer. Based on the standard deviation of the MDmax and MDmin from the 30-year mean recorded at NW sites (Chapter 5), it was decided a MDmin or MDmax that differed from the 30-year mean by more than 3°C would be considered significantly different from the norm, whilst a monthly rainfall total that differed from the 30-year mean by more

than 50%, was considered significant. All temperatures are reported to the nearest 0.1 °C

4.2.3 Summary of climate data

2010 was generally cooler than the 30-year mean in the winter months (January, February, November and December), with a lower MDmax (**Error! Reference source not found.**) and MDmin (Figure 4.2), and warmer than average through the rest of the year, with a greater MDmax and MDmin compared to the 30-year mean. The MDmax in January and December 2010 was 3.1 and 5.3 °C lower than the 30-year mean respectively. In April, May, June and October 2010, the MDmax was greater than the 30-year mean by 4.3, 3.3, 5.0 and 5.7 °C respectively. In all other months in 2010, the MDmax was within 3 °C of the 30-year mean. The MDmin in June and July 2010 was greater than the 30-year mean by 3.5 and 3.0 °C respectively. In December 2010 the MDmin was 5.4 °C cooler than the 30-year mean. In the other nine months in 2010 the MDmin was within 3 °C of the 30-year mean.

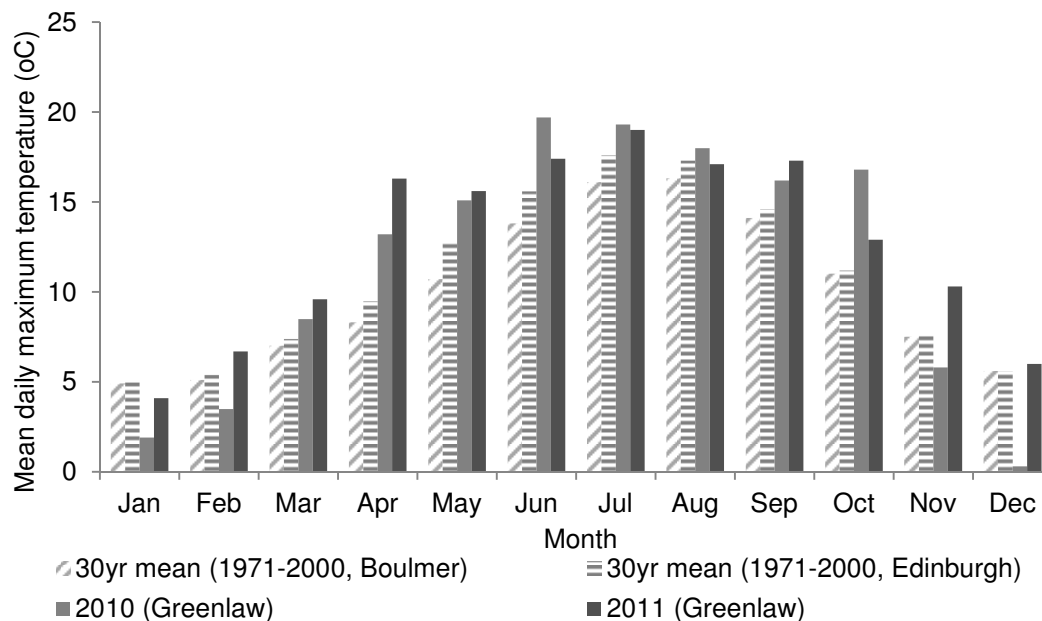


Figure 4. Mean daily maximum temperature (MDmax) in each month in 2010 and 2011 recorded at Greenlaw met. station, alongside the 30-year (1971-2000) mean daily maximum temperature recorded at Edinburgh and Boulmer met. stations (adjusted for altitude as described in the text)

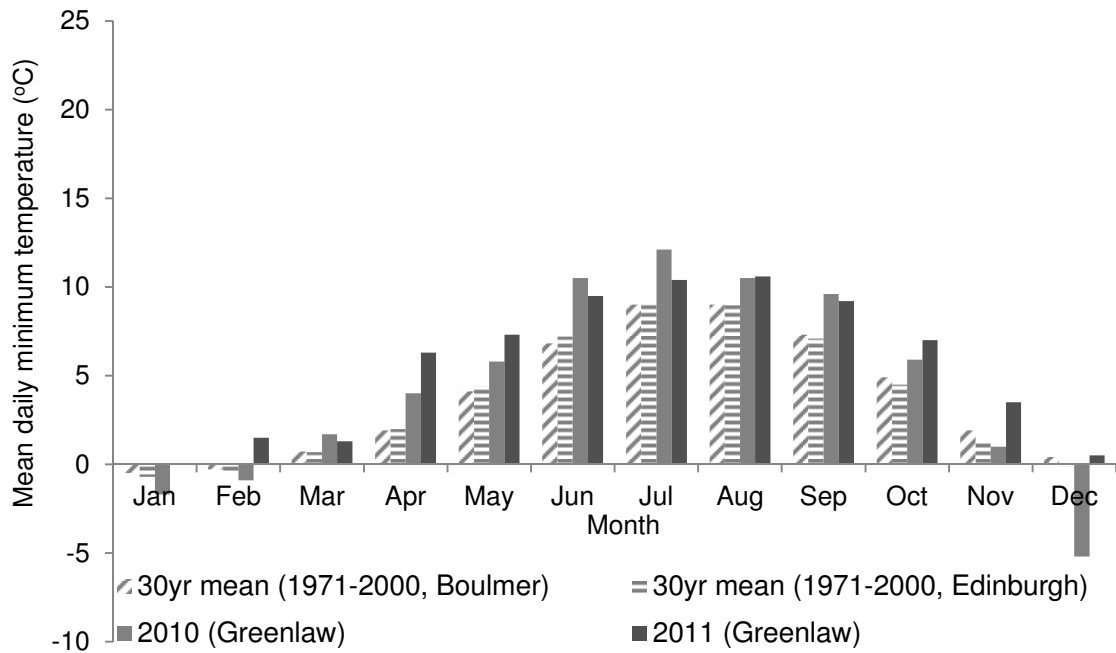


Figure 4.2 Mean daily minimum temperature (MDmin). in each month in 2010 and 2011 recorded at Greenlaw met. station, alongside the 30-year (1971-200) mean minimum. daily temperature recorded at Edinburgh and Boulmer met. stations (adjusted for altitude as described in text)

2011 tended to be hotter than the 30-year mean throughout the year. The MDmax in April and May 2011 was greater than the 30-year mean by 7.4 and 3.8 °C respectively, whilst for the other 10 months, the MDmax was within 3 °C of the 30-year mean. The MDmin in April and May 2011 was greater than the 30-year mean by 4.3 and 3.0 °C respectively. In all other months of 2011 the MDmin was within 3 °C of the 30-year mean.

The year 2010 began with an average level of rainfall in January, whilst February and March 2010 were both slightly wetter than average, with rainfall totals of 148% and 152% respectively of the 30-year mean (Figure 4.3). The first part of the 2010 growing season (April, May and June) was drier than average, rainfall totals were 38.8%, 49% and 52.9% of the 30-year mean. The monthly mean rainfall totals in July-October 2010 were all well within 50% of the 30-year means. 2010 ended with a wetter than average November and a drier than average December, with rainfall totals of 156% and 50.1% the of the 30-year mean. The average rainfall total for the region in 2010 was 802.7 mm compared to a 30-year mean of 805 mm.

Chapter 4 Results and discussion from Scottish sites

In 2011, February and August were significantly wetter than average, receiving 159 and 160% of their 30-year mean rainfall totals respectively. The growing season in 2011 begun with a drier than average March and April, which received 41.6 and 15.8% respectively of their 30-year mean rainfall totals. November 2011 was also significantly drier than average, receiving 49.8% of the 30-year mean rainfall total. The averaged rainfall total for the region in 2011 was 719 mm.

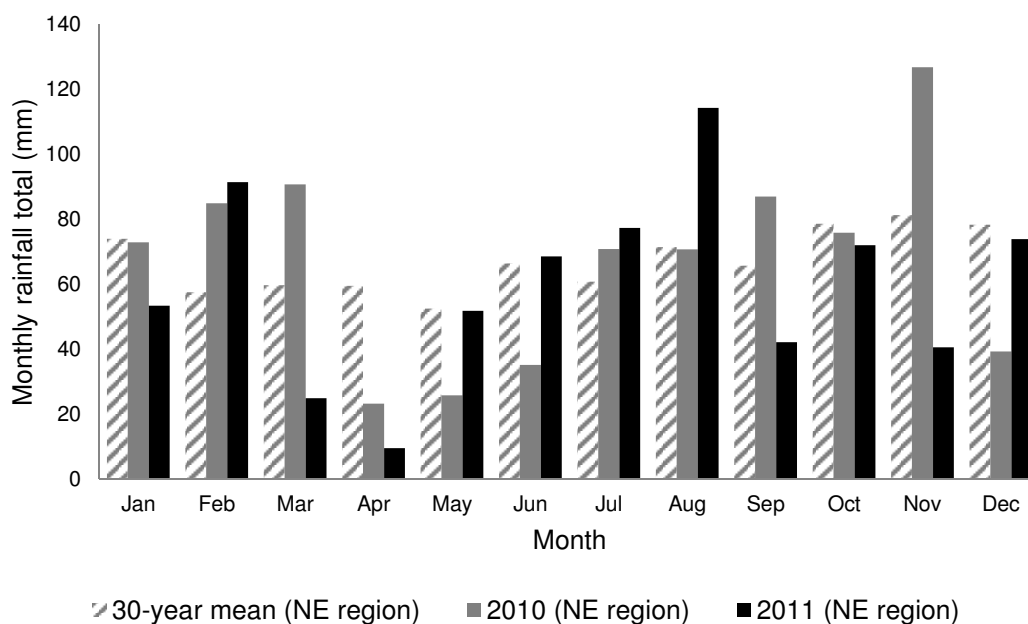


Figure 4.3 Monthly rainfall total in 2010 and 2011 averaged for the NE England climatic region, alongside the 30-year mean monthly rainfall total for the same region, data obtained from UK Met. Office

4.3 Soil chemical data

This section presents the results of the soil chemical analysis carried out on soil samples from the Scottish sites, as described in section 3.6. In total four Scottish sites, each containing an IM plot and paired SRG plot that was established in the previous 10 years, were sampled. The sites (S3, S5, S8, and S9) are named according to the age of the SRG (in years) in 2010. The data presented includes results from analysis of soil samples for available N, total N, total P, total C, OM, CN ratio and pH. The different ages of the SRG plots studied meant that they formed a

chronosequence. The differences in the mean values of each measured soil variable between paired IM and SRG plots were plotted against the age of the SRG plot to determine whether there was any obvious trend with age of the SRG. There was only a significant relationship for one variable (BD).

4.3.1 Analysis of soil chemical data

Initial data inspection was carried out to identify whether any correlation existed between the age of the SRG and the difference between the paired IM and SRG sites for any of the measured parameters. No such correlation was found, most likely because the site pairs differed from one another in many respects other than age of the SRG, including soil type, location, slope and management history. Analysis was then carried out to identify any significant effects of management type (IM / SRG) on any of the measured soil chemical properties. The mean parameter values from the five sampling points in each plot (from here after referred to as the 'plot mean') were analysed using GENSTAT14 software. Due to the high inter-site variation, a randomized block ANOVA design was used, with the data blocked according to the site pair (S3, S5, S8 and S9), to reduce the noise in the datasets due to site differences. Separate models were written for the spring (March / April) and summer (July) data. The spring data were analysed using a split plot design split two ways, first for soil depth (0-10 cm / 30-40 cm) then for the year (2010 / 2011). The summer soil samples were taken from 0-10 cm depth only, so the ANOVA design was split one way.

The data for NO_3^- -N, NH_4^+ -N and total available N (NO_3^- -N + NH_4^+ -N) concentration were found not to be normally distributed so the natural log of the value was taken prior to carrying out the ANOVA. In addition 1 was added to each of the values for NH_4^+ -N concentration to remove negative values. The values for the ratio of available NH_4^+ -N: NO_3^- -N from the spring samplings were increased by 1 and then transformed using a Box-Cox transformation to normalise the data distribution, the ratio of the concentrations of NH_4^+ -N: NO_3^- -N measured in the summer samples were increased by 1 and transformed by taking the natural logarithm.

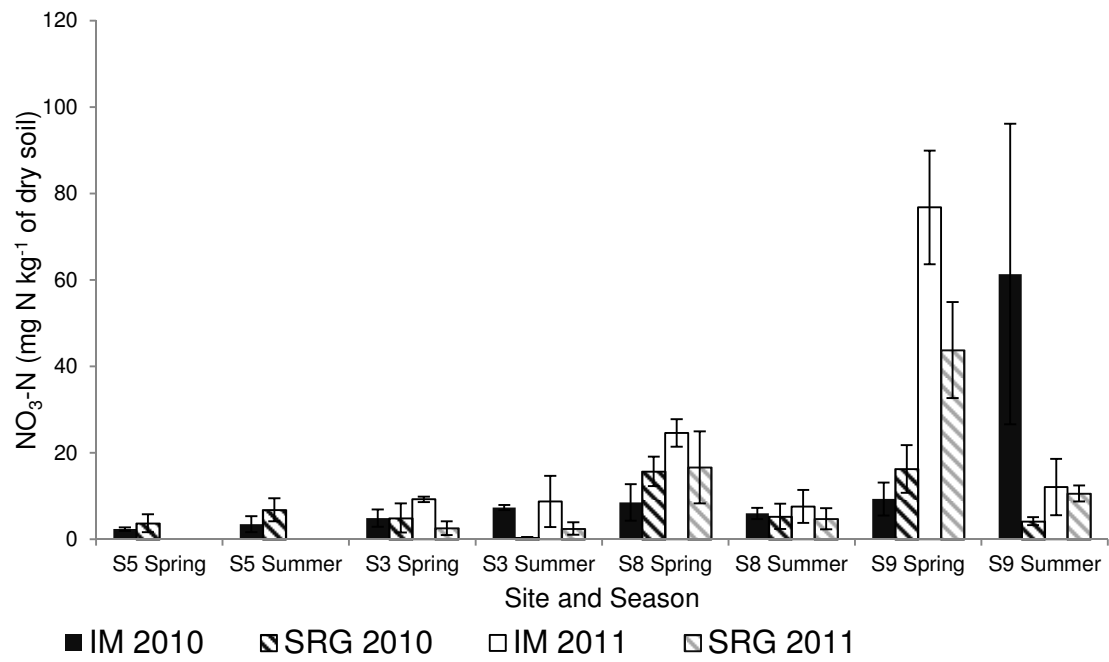
4.3.2 Soil nitrogen

The mean NO_3^- -N concentration (this includes NO_2^- -N, usually present at very low concentrations in soil, as explained in section 3.6.6) measured on each occasion at sites S3 and S5 and in both summers at site S8, were below 10 mg N kg^{-1} of dry soil at both depth ranges (0-10 and 30-40 cm), and showed no consistent effect of management type (Figure 4.4). The mean soil NO_3^- -N concentration at Site S8 was higher in spring compared to summer in 2010 and 2011, but again there was no consistent relationship with management type. The mean soil NO_3^- -N concentration at site S9 was $< 20 \text{ mg N kg}^{-1}$ of dry soil in spring 2010 and summer 2011 with similar concentrations measured at both depth ranges in the spring.

At site S9 in spring 2011 the mean measured NO_3^- -N concentration in the 0-10 cm depth range was $> 40 \text{ mg N kg}^{-1}$ of dry soil under both management types but was higher in the IM plot, with a mean concentration of $76.8 (\pm 3.83 \text{ mg N kg}^{-1} \text{ of dry soil})$ and $43.7 (\pm 2.45 \text{ mg N kg}^{-1} \text{ of dry soil})$ in the IM and SRG plots, respectively. At the 30-40 cm depth range the mean NO_3^- -N concentration was again larger in the IM compared to the SRG plots, with mean concentrations of $48.2 (\pm 3.49)$ and $10.6 (\pm 3.99 \text{ mg N kg}^{-1} \text{ of dry soil})$, respectively. In summer 2010 the mean NO_3^- -N concentration (0-10 cm) in the IM plot of $61.4 (\pm 48.2 \text{ mg N kg}^{-1} \text{ of dry soil})$ was more than 14 times greater than the mean NO_3^- -N concentration of $4.15 (\pm 10.6 \text{ mg N kg}^{-1} \text{ of dry soil})$ in the SRG plot.

Chapter 4 Results and discussion from Scottish sites

a)



b)

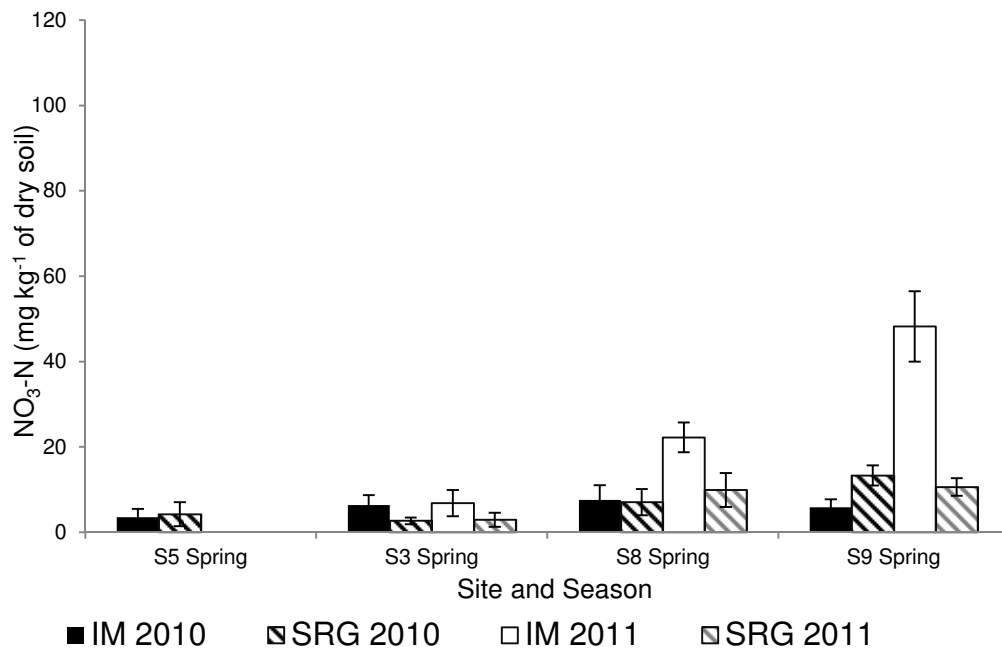


Figure 4.4 Mean ($n=5$) $\text{NO}_3\text{-N}$, in soil samples taken from each paired IM (plain bars) and SRG (speckled bars) plot at the four Scottish field sites (S3, S5, S8, S9), from a) 0-10 cm depth and b) 30-40 cm depth. Error bars show $\pm 1\text{SD}$.

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Despite these differences between the management types at individual sites on some sampling occasions, when the data from all sites were combined, there was no clear difference in soil NO_3^- -N concentration between the IM and SRG plots when sampled in the spring. An ANOVA (Section 4.2) conducted on the combined NO_3^- -N concentration data from all four sites in spring 2010 / 2011 (Figure 4.5), showed no significant difference in mean ($n=16$) NO_3^- -N concentration between the IM and SRG plots ($F(1,3)=5.35$, $P=0.104$). There appears to be a slight trend for higher NO_3^- -N concentrations in the IM plots in summer (Figure 4.6), but an ANOVA showed that this was not statistically significant ($F(1,3)=0.56$, $P=0.509$).

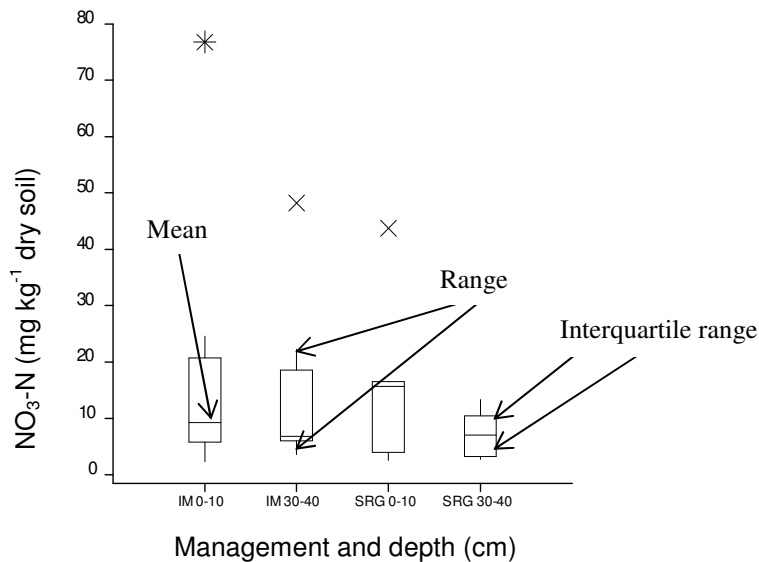


Figure 4.5 Box plot summarising the distribution of the 'plot means' for soil available NO_3^- -N concentration for IM and SRG plots at sites S3, S5, S8 and S9, showing median, inter-quartile range (IQ range), and range of the 'plot mean' values in spring 2010 and 2011. Data grouped according to plot management type (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm). Values lying > 1.5 times inter-quartile range (cross marker) or > 3 times the inter-quartile range (star marker) outside of the quartiles are shown as outliers.

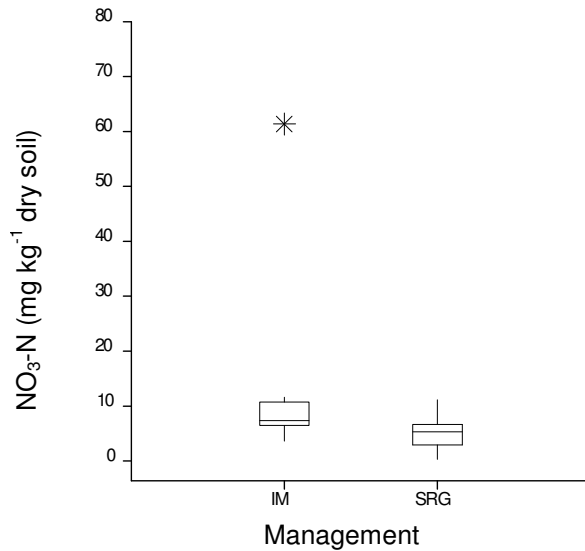


Figure 4.6 Box plot, summarising the distribution of the 'plot means' for NO_3^- -N concentration, showing median, inter-quartile range, and range. Samples collected from 0-10 cm depth in summer 2010 and 2011. Data grouped according to plot management (IM/SRG). Values lying > 1.5 times the inter-quartile range (cross marker) or > 3 times the inter-quartile range (star marker) outside of the quartiles are shown as outliers.

The mean NH_4^+ -N concentrations measured in the soil samples were generally lower than the NO_3^- -N concentrations, ranging between $0.303 (\pm 0.787)$ and $12.3 (\pm 2.17 \text{ mg kg}^{-1} \text{ of dry soil})$ in the 0-10 cm depth range and 0 and $11.7 (\pm 0.751 \text{ mg kg}^{-1} \text{ of dry soil})$ in the 30-40 cm depth range (Figure 4.7). There was a relatively large amount of variation within plots, resulting in high standard deviations from the mean. On most occasions the mean soil NH_4^+ -N concentrations under the two management types IM / SRG at each site were within 1 SD of each other, apart from in spring 2010, where in the 0-10 cm depth range at site S8, the mean NH_4^+ -N concentration in the IM plot was greater than in the SRG plot. In summer 2010 the mean NH_4^+ -N concentration in the 0-10 cm depth range was greater in the SRG plot compared to the IM plot at sites S3, S8 and S9. The mean soil NH_4^+ -N concentration was also greater in the SRG plot than the IM plot in the 30-40 cm depth range at site S3 in spring 2011.

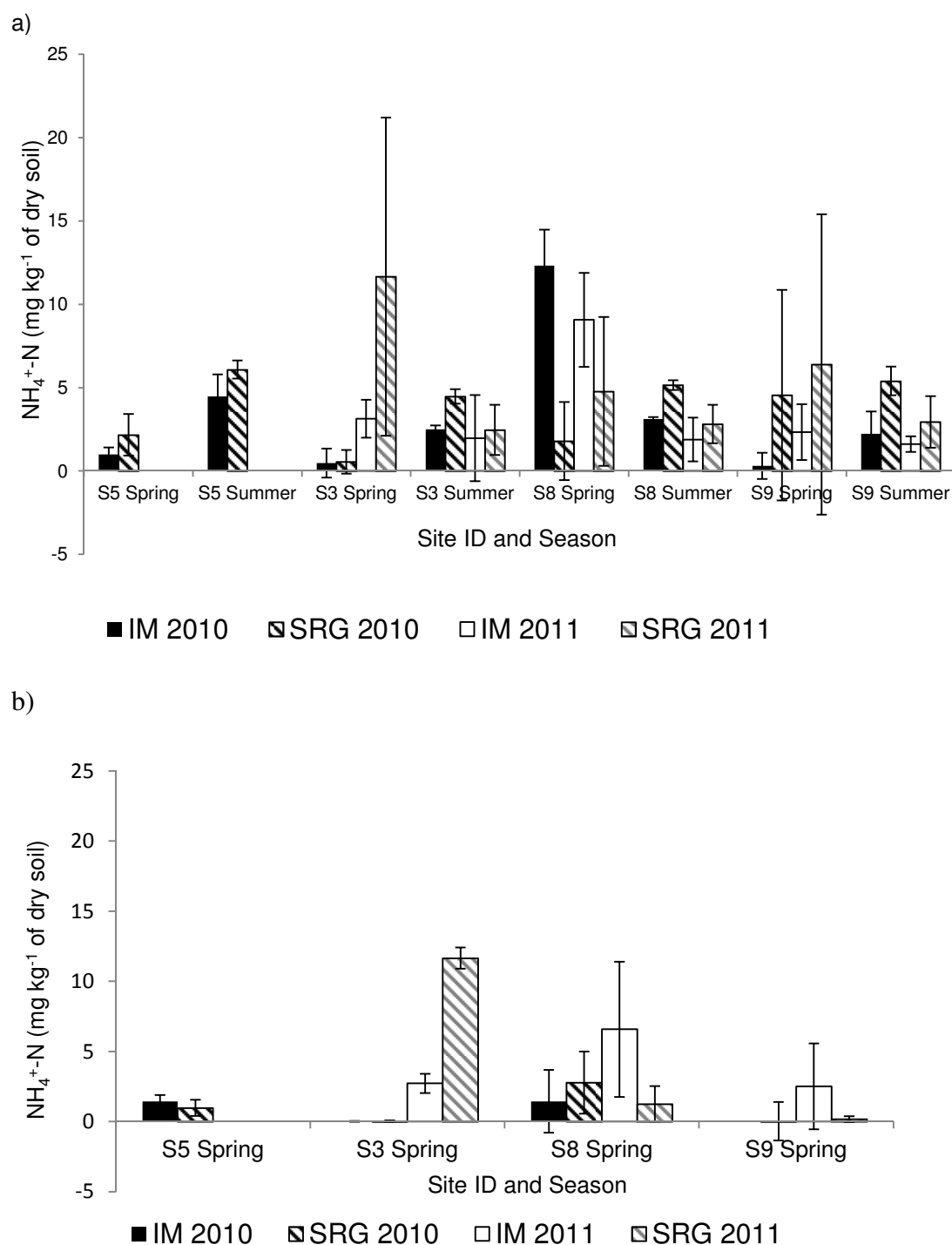


Figure 4.7 Mean ($n=5$) $\text{NH}_4^+\text{-N}$, soil samples taken from each paired IM (plain bars) and SRG (speckled bars) plot at the four field sites (S3, S5, S8, S9). Soil samples taken from a) 0-10 cm depth and b) 30-40 cm depth. Error bars show $\pm 1\text{SD}$.

When the $\text{NH}_4^+\text{-N}$ concentration data from all sites were combined, there appeared to be a tendency for slightly higher and a greater range of concentrations in the 0-10 cm depth range (Figure 4.8). In samples collected in the summer the $\text{NH}_4^+\text{-N}$

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concentration appears to have a tendency to be higher in the SRG plots compared to the IM plots (Figure 4.9). As with the NO_3^- -N data, despite suggestions of a trend and the differences in NH_4^+ -N concentration observed between paired plots on some occasions, an ANOVA carried out on the NH_4^+ -N concentration data showed no significant difference in mean ($n=16$) NH_4^+ -N concentration between the IM and SRG plots ($F(1,3)=0.07$, $P=0.813$) for the spring samples nor for the summer soil samples ($F(1,3)=3.20$, $P=0.171$)

With the exception of three outliers, the mean ratio of available NH_4^+ -N: NO_3^- -N in all the spring soil samples was < 1 . There was no significant difference in the ratio of available soil NH_4^+ -N: NO_3^- -N between the two management types ($F(1,3)=0.56$, $P=0.508$) (Figure 4.10). The ratio of available soil NH_4^+ -N: NO_3^- -N in samples collected in the summer seemed to be higher in samples from the SRG plots (Figure 4.11), however this difference was not statistically significant ($F(1,3)=1.17$, $P=0.359$).

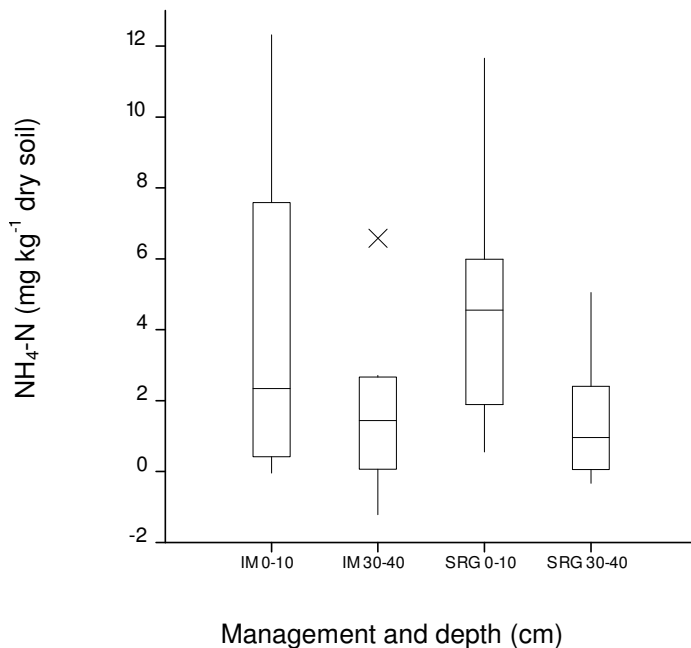


Figure 4.8 Box plot summarising the distribution of the 'plot means' for soil available NH_4 -N concentration, showing median, inter-quartile range, and range in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and

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30-40 cm). Values lying > 1.5 times the inter-quartile range outside of the quartiles are shown as outliers

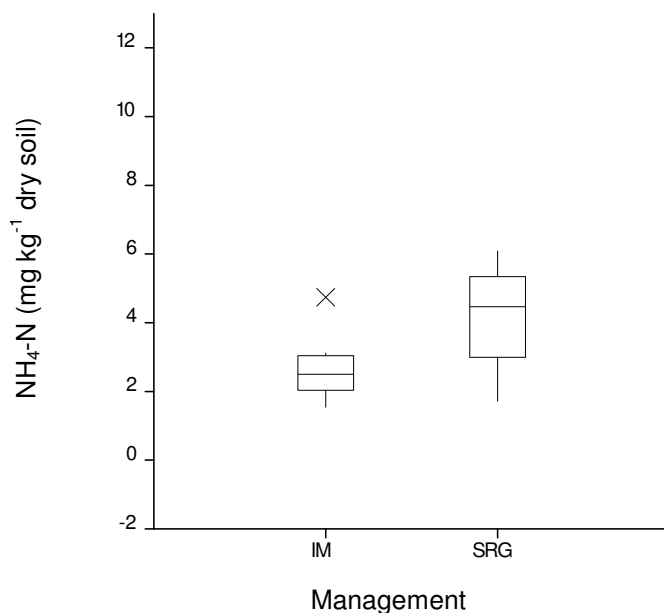


Figure 4.9 Box plot summarising the distribution of the 'plot means' for soil available $\text{NH}_4\text{-N}$ concentration, showing median, inter-quartile range, and range. Samples collected from 0-10 cm depth in summer 2010 and 2011. Data grouped according to plot management (IM/SRG). Values lying > 1.5 times the inter-quartile range outside of the quartiles are shown as outliers

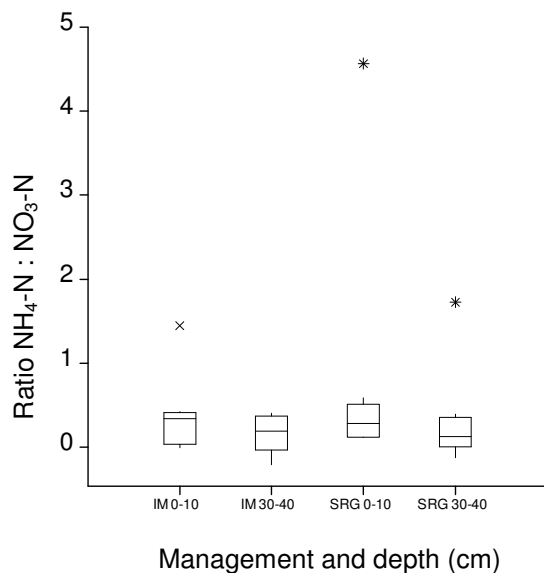


Figure 4.10 Box plot summarising the distribution of the 'plot means' for the ratio of soil available $\text{NH}_4^+\text{-N} : \text{NO}_3^-\text{-N}$, showing median, inter-quartile range, and range in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm). Values lying > 1.5 times the inter-quartile range (cross marker) or > 3 times the inter-quartile range (star marker) outside of the quartiles are shown as outliers.

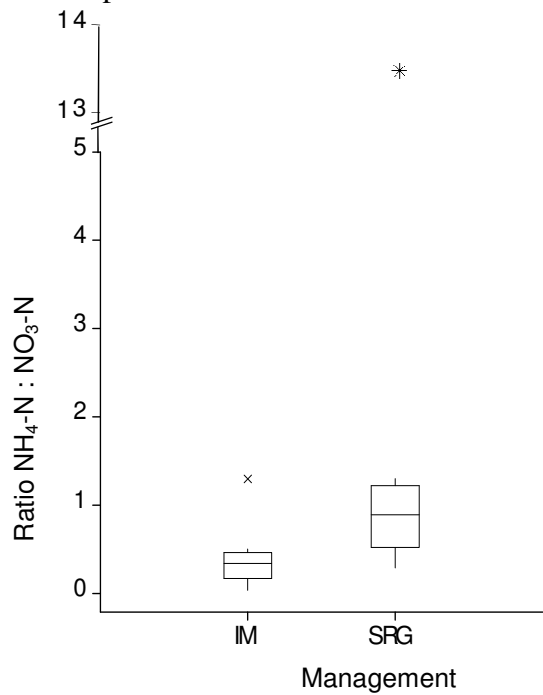


Figure 4.11 Box plot summarising the distribution of the 'plot means' for the ratio of soil available $\text{NH}_4^+\text{-N}:\text{NO}_3^-\text{-N}$, showing median, inter-quartile range, and range in for samples collected in summer 2010 and 2011. Data grouped according to plot management (IM/SRG). Values lying > 1.5 times the inter-quartile range (cross marker) or > 3 times the inter-quartile range (star marker) outside of the quartiles are shown as outliers.

There is little sign of any influence of management type or depth range on the mean concentrations of soil available N ($\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}$) measured (Figure 4.12).

ANOVAs (Section 4.2) carried out on the mean ($n=16$) concentrations of available N, showed no significant difference between the IM and SRG plots in the spring soil samples ($F(1,3)=5.80$, $P=0.095$), nor for the summer soil samples (Figure 4.13) ($F(1,3)=0.43$, $P=0.560$)

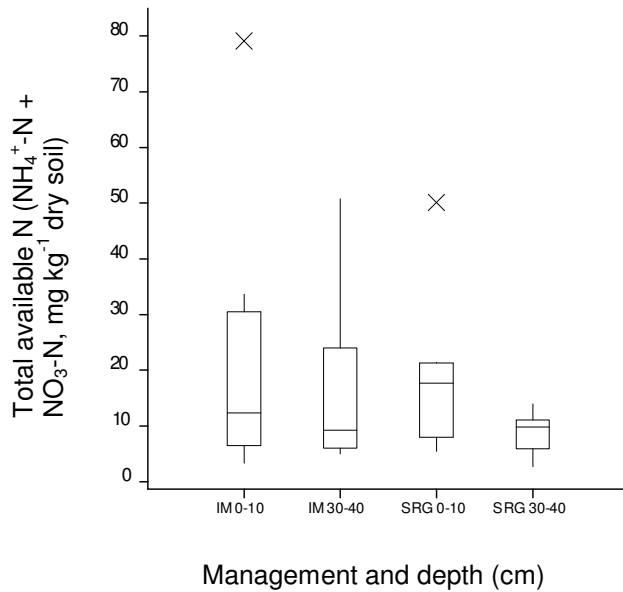


Figure 4.12 Box plot summarising the distribution of the 'plot means' (n=5) for soil available N (NO₃⁻-N + NH₄⁺-N) concentration, showing median, inter-quartile range, and range. Samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm). Values lying > 1.5x inter-quartile range outside of the quartiles are shown as outliers

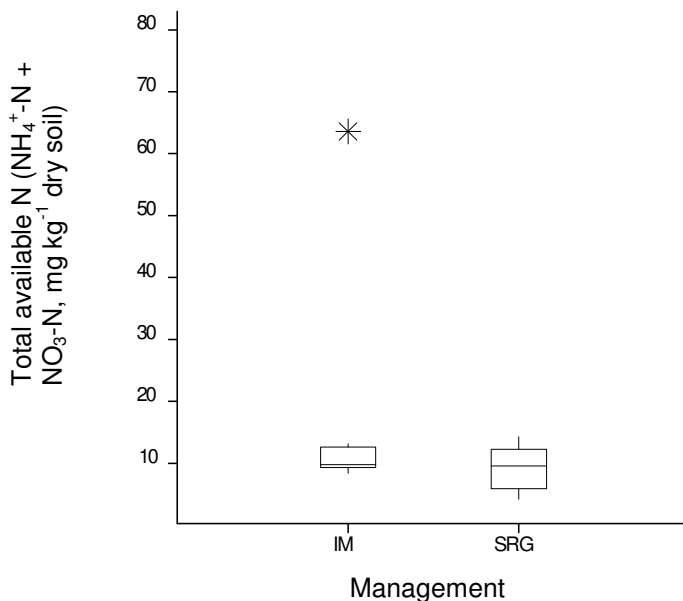


Figure 4.13 Box plot summarising the distribution of the 'plot means' (n=5) for soil available N (NO₃⁻-N + NH₄⁺-N) concentration, showing median, inter-quartile range, and range. Samples collected from 0-10 cm depth in summer 2010 and 2011. Data grouped according to plot management (IM/SRG). Values lying > 1.5 times the inter-quartile range outside of the quartiles are shown as outliers

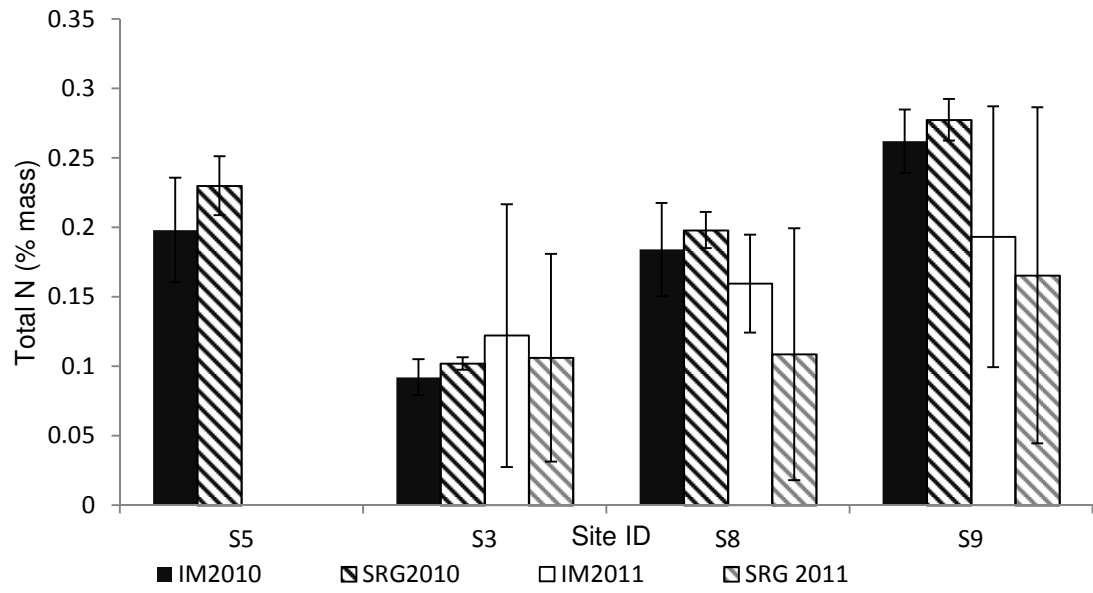
As a % of the total N concentration the available N concentration was on most occasions less than 1 and never more than 4.1% (Table 4.1). The mean total N concentration measured in the soil samples varied very little between the two management types (IM / SRG) at a given site and occasion (Figure 4.14). There were some differences between sites with total soil N concentration tending to be lower in S3, ranging from 0.086 to 0.122% mass across both depth ranges. The site with the greatest soil total N concentration (0.27% mass) was Site S9. The total N concentrations were similar at both depth ranges and the within plot variation was greater in 2011 than 2010, but overall the mean total N concentrations recorded across all sites in 2010 and 2011 varied little, in either magnitude or distribution, between either management type or sampling date. An ANOVA carried out on the mean total soil N concentrations in 2010/2011 (spring) (Figure 4.15), showed no significant difference in mean between the IM and SRG plots ($F(1,3)=0.12$, $P=0.754$).

Table 4.1 Measured concentration of available soil N (NO_3^- -N + NH_4^+ -N) as a % of the measured concentration of total soil N for each plot in the spring of 2010 and 2011.

Year	Depth range (cm)	Mean available N concentration (NO_3^- -N + NH_4^+ -N) as a % of mean total N concentration for each plot							
		S5 IM	S5 SRG	S3 IM	S3 SRG	S8IM	S8SRG	S9IM	S9 SRG
2010	0-10	0.167	0.255	0.582	0.534	1.13	0.883	0.366	0.749
	30-40	0.259	0.295	0.715	0.315	0.573	0.622	0.230	0.494
2011	0-10	NA	NA	1.01	1.34	2.11	1.97	4.10	3.03
	30-40	NA	NA	1.01	2.18	1.31	0.586	3.09	0.470

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a)



b)

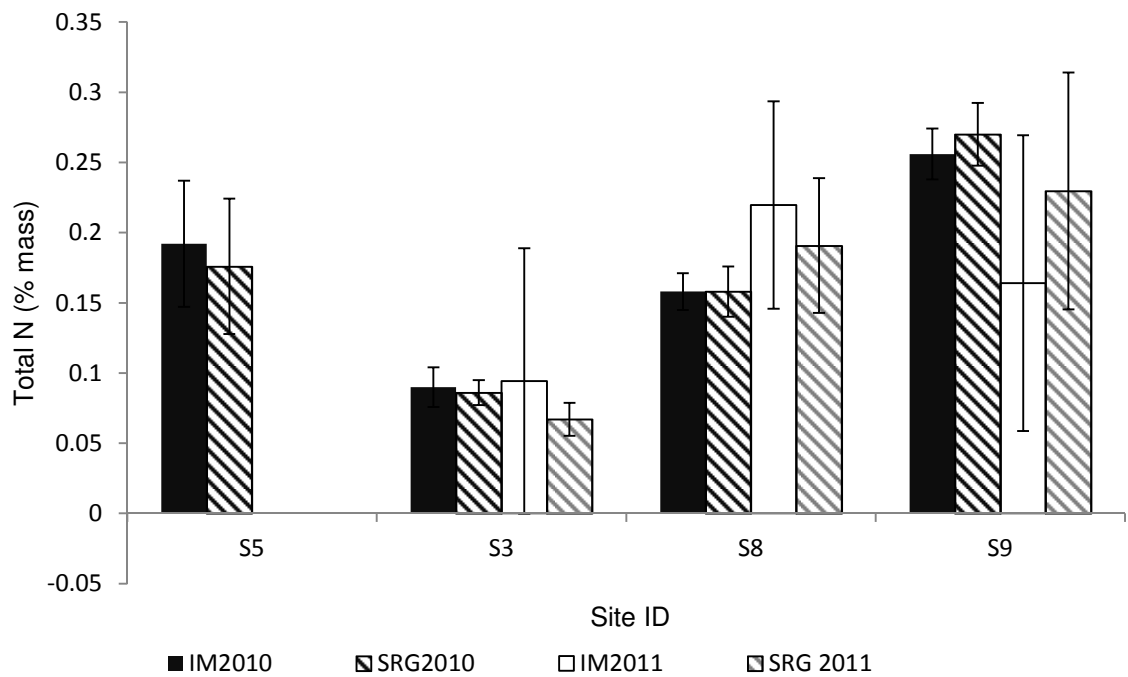


Figure 4.14 Mean ($n=5$) total N content of soil samples taken from each paired IM (plain bars) and SRG (speckled bars) plot at the four field sites (S3, S5, S8, S9). Soil samples taken from a) 0-10 cm depth and b) 30-40 cm depth. Error bars show ± 1 SD.

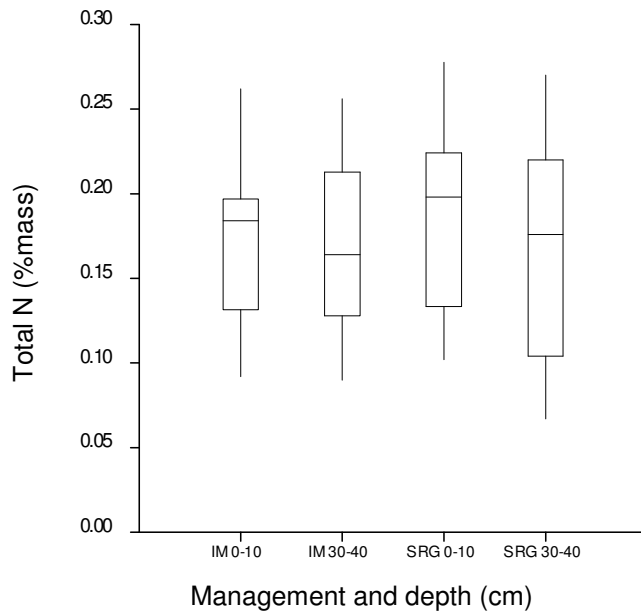


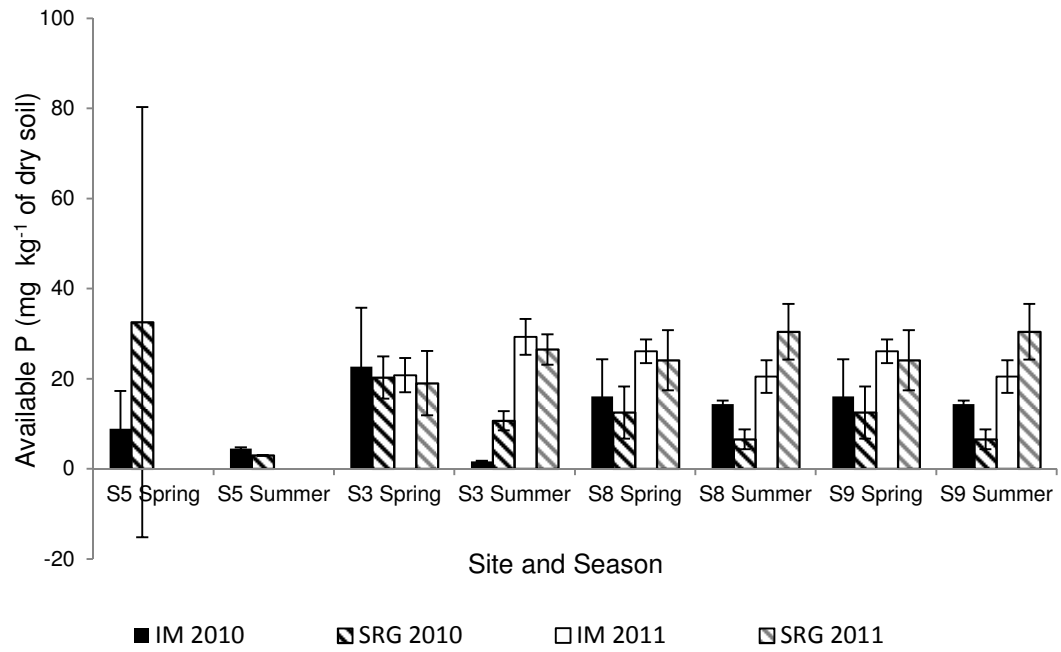
Figure 4.15 Box plot summarising the distribution of the 'plot means' for total soil N content, showing median, inter-quartile range, and range of samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm).

4.3.3 Soil P

The mean available soil P concentrations were generally very similar between paired IM and SRG plots for a given site and occasion (Figure 4.16). At site S3 in summer 2010 in the 0-10 cm depth range (Figure 4.16a), the mean available P concentration of $1.63 (\pm 0.16 \text{ mg kg}^{-1})$ of dry soil) in the SRG plot was more than 6.5 times greater than the mean available P of $10.7 (\pm 2.13 \text{ mg kg}^{-1})$ of dry soil) in the IM plot, whilst the mean available P was higher in the IM plot compared to the SRG plot in summer 2010 at sites S8 and S9. In the 30-40 cm depth range (Figure 4.16b) in spring 2011 at site S9 the mean available P concentration of $46.2 (\pm 2.96 \text{ mg kg}^{-1})$ of dry soil) in the IM plot was more than two times the mean concentration of $21.9 (\pm 4.31 \text{ mg kg}^{-1})$ of dry soil) in the SRG plot. An ANOVA carried out on the concentrations of available P in spring 2010 / 2011 (Figure 4.17), showed no significant difference in mean P concentration between the IM and SRG plots ($F(1,3)=0.03$, $P=0.876$). An ANOVA carried out on the mean available P in the summer samples (Figure 4.18) also showed no significant difference between the two management types, ($F(1,3)=1.18$, $P=0.357$).

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a)



b)

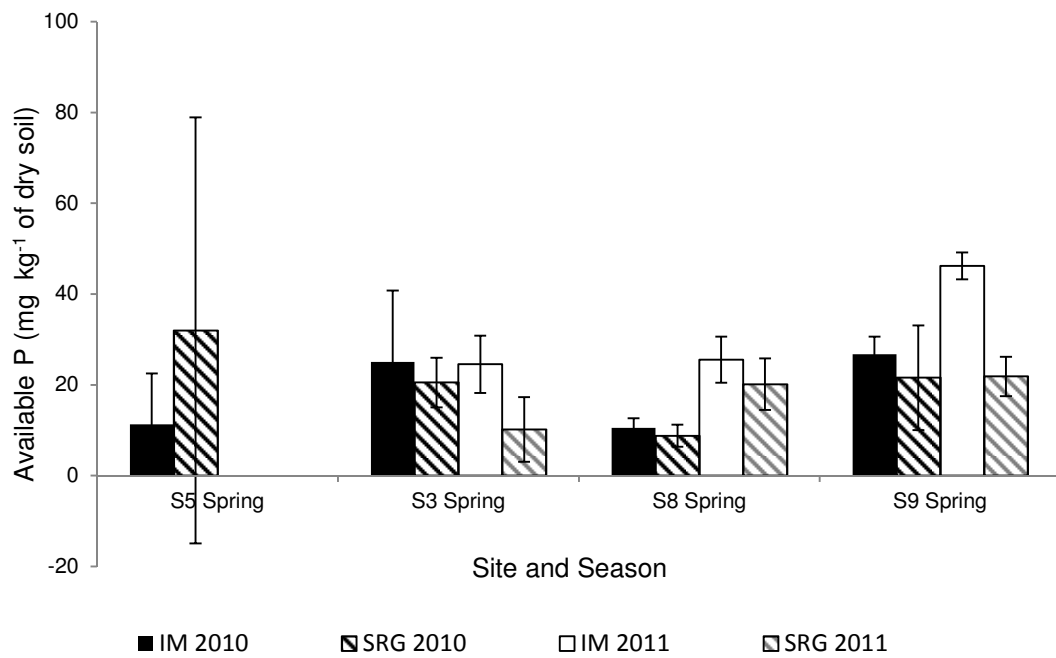


Figure 4.16 Mean (n=5) available P concentration of soil samples taken from each paired IM (plain bars) and SRG (speckled bars) plot) at the four field sites (S3, S5, S8, S9). Soil samples taken from a) 0-10 cm depth and b) 30-40 cm depth. Error bars show ± 1 SD.

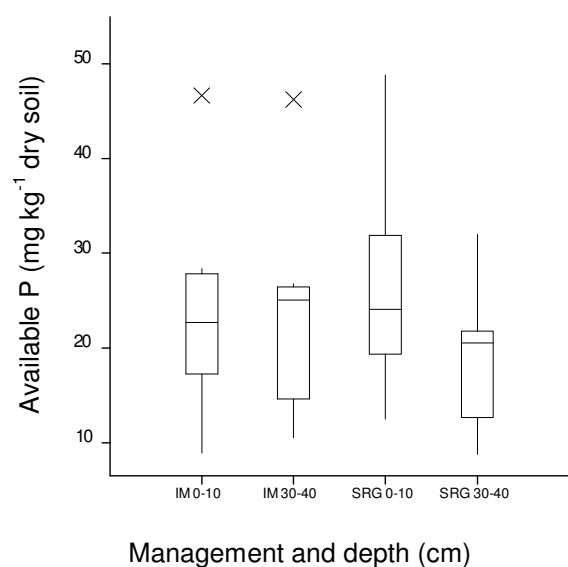


Figure 4.17 Box plot summarising the distribution of the 'plot means' for available P concentration showing median, inter-quartile range, and range for samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm). Values lying > 1.5 times the inter-quartile range outside of the quartiles are shown as outliers.

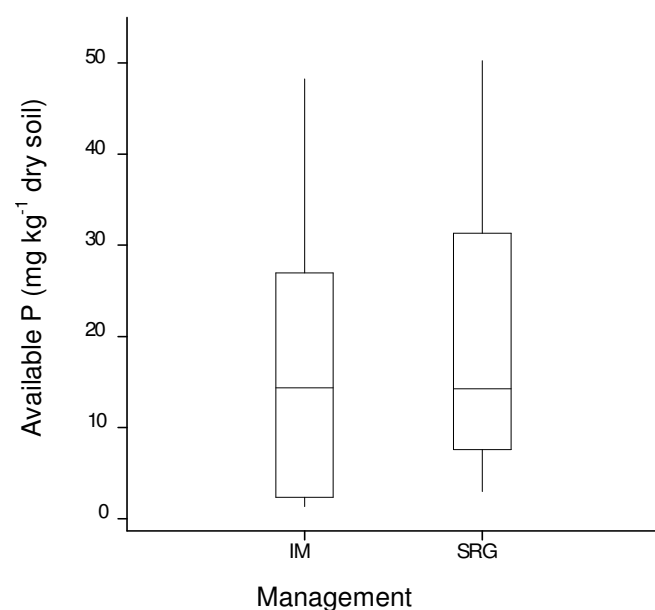


Figure 4.18 Box plot summarising the distribution of the 'plot means' for soil available P concentration, showing median, inter-quartile range, and range for samples collected in summer 2010 and 2011 from 0-10 cm depth. Data grouped according to plot management (IM/SRG).

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As a % of the total measured P concentration, the measured available P concentration was on most occasions less than 14%, except for at site S3 in 2010. In 2010 the mean available P concentration in plot S3 IM was 29.9 and 30.6% of the mean total P concentration measured in the 0-10 and 30-40 cm depth ranges, respectively. In plot S3 SRG, the mean available P concentration in 2010 was 17.2 and 27.7% of the mean total P concentration in the 0-10 and 30-40 cm depth range respectively (Table 4.2).

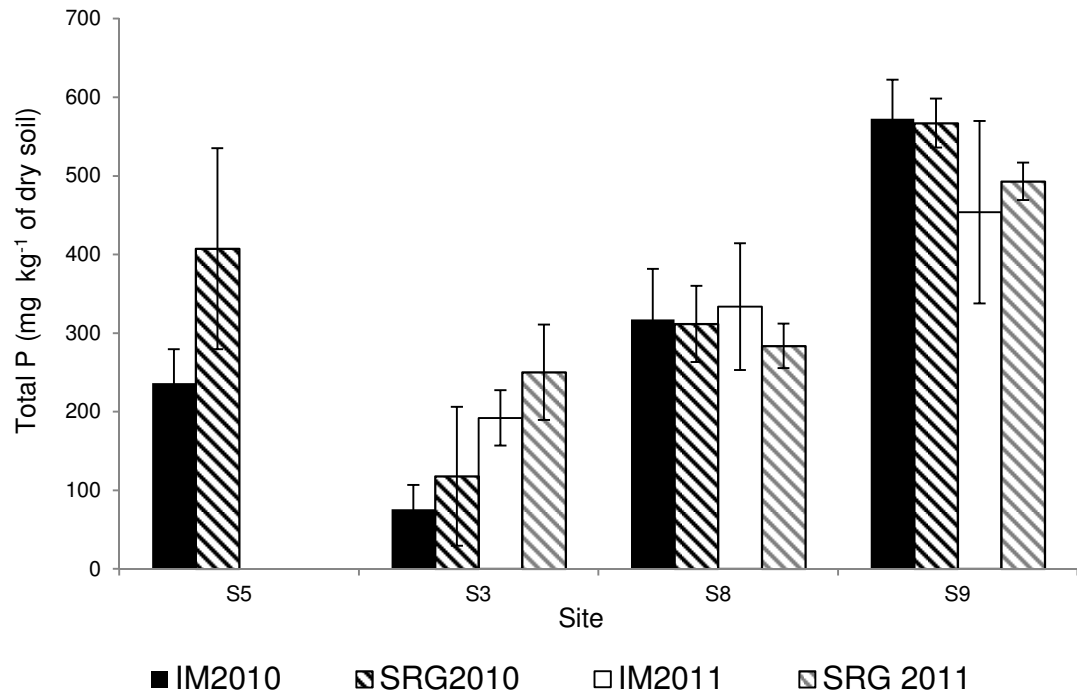
There was little variation between paired IM and SRG plots in mean concentration of total P measured in the soil samples (Figure 4.19). The range and distribution of the plot means for total soil P concentration were very similar for both management types and depth ranges (Figure 4.20). The total soil P concentration tended to be lower in site S3, ranging from 74.1 (± 27.8) to 250 (± 28.2 mg kg⁻¹ of dry soil) across both depth ranges, and higher in S9, ranging from 439 (± 95.9) to 573 (± 49.7 mg kg⁻¹ of dry soil) across both depth ranges. An ANOVA carried out on the soil total P concentrations in 2010 / 2011, showed no significant difference in mean concentration of total P between the IM and SRG plots ($F(1,3)=1.10$, $P=0.371$).

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Table 4.2 Measured concentration of soil available P as a % of the measured concentration of soil total P for each plot in the spring of 2010 and 2011

Year	Depth range (cm)	Mean available P concentration as a % of total P concentration for each plot							
		S5 IM	S5 SRG	S3IM	S3 SRG	S8 IM	S8 SRG	S9 IM	S9 SRG
2010	0-10	3.77	7.99	29.9	17.2	5.07	4.01	2.81	2.20
	30-40	5.79	9.93	30.6	27.7	3.68	3.89	4.96	3.94
2011	0-10			10.8	7.60	7.82	8.49	5.75	4.89
	30-40			13.3	5.24	10.5	6.64	9.39	4.98

a)



b)

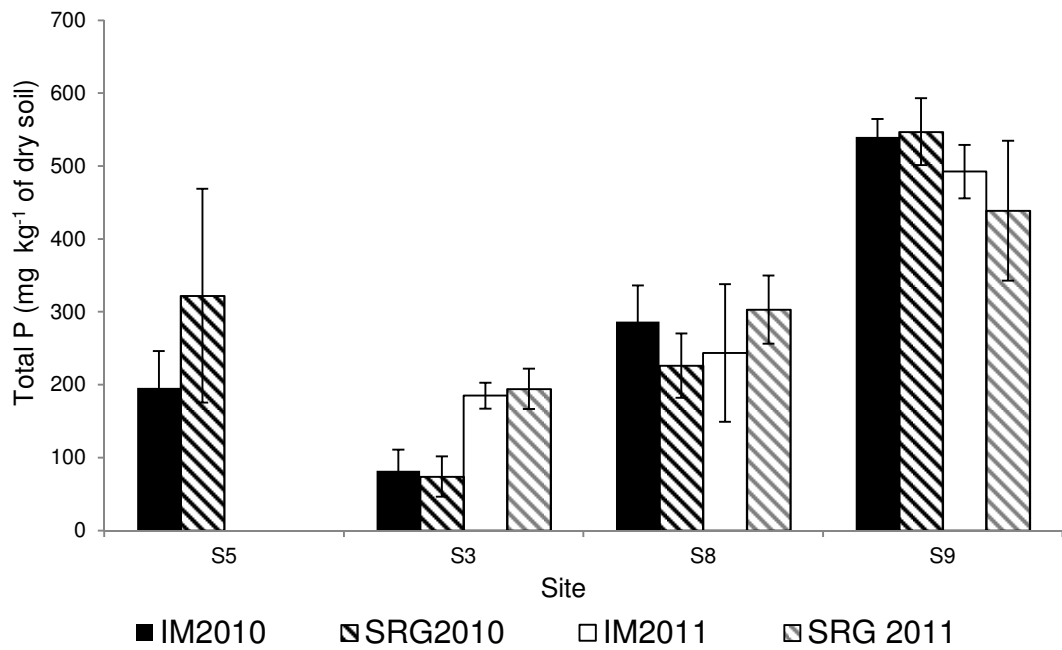


Figure 4.19 Mean (n=5) total P content of the five soil samples taken from each paired IM (plain bars) and SRG (speckled bars) plot (plot mean) at the four field sites (S3, S5, S8, S9), for soil samples taken from a) 0-10 cm depth and b) 30-40 cm depth. Error bars show ± 1 SD from the mean.

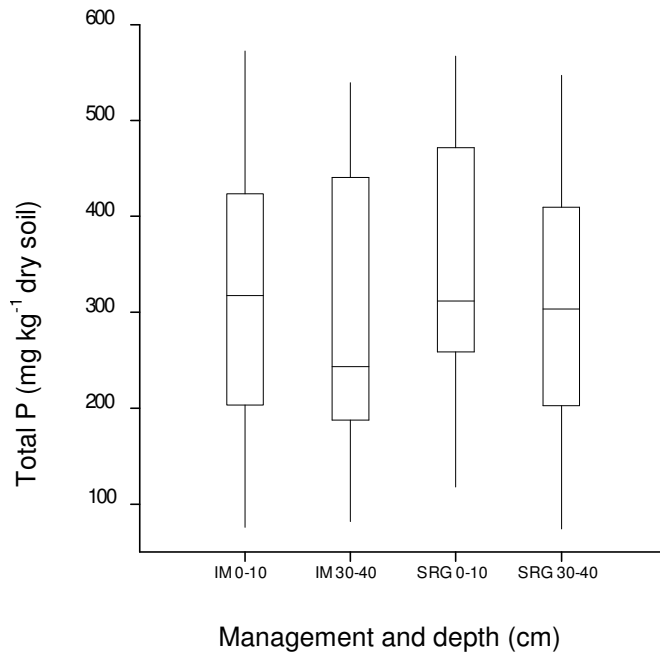


Figure 4.20 Box plot summarising the distribution of the 'plot means' for soil total P concentration, showing median, inter-quartile range, and range for samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm).

4.3.1 Soil C:N ratio

The mean C:N ratio in all plots over both depth ranges and sampling occasions varied very little, ranging between 9.7 and 11.4, except for two outliers, with most plot means lying between 10.5 and 11.4 (Figure 4.21). An ANOVA conducted on the mean C:N ratios in 2010 and 2011 (spring) combined, showed no significant difference in mean C:N ratio between the IM and SRG plots ($F(1,3)=2.12$, $P=0.241$)

4.3.2 Soil organic matter concentration

The mean soil organic matter concentration in each plot across both depth ranges and sampling occasions ranged between 2.03 and 8.05% mass dry soil. The median and range of the 'plot means' for soil organic matter concentration varied very little between the two management types and depth ranges (Figure 4.22). An ANOVA carried out on the soil organic matter concentrations in samples from spring 2010 and 2011 combined showed no significant difference in mean C:N ratio between the IM and SRG plots ($F(1,3)=8.12$, $P=0.065$).

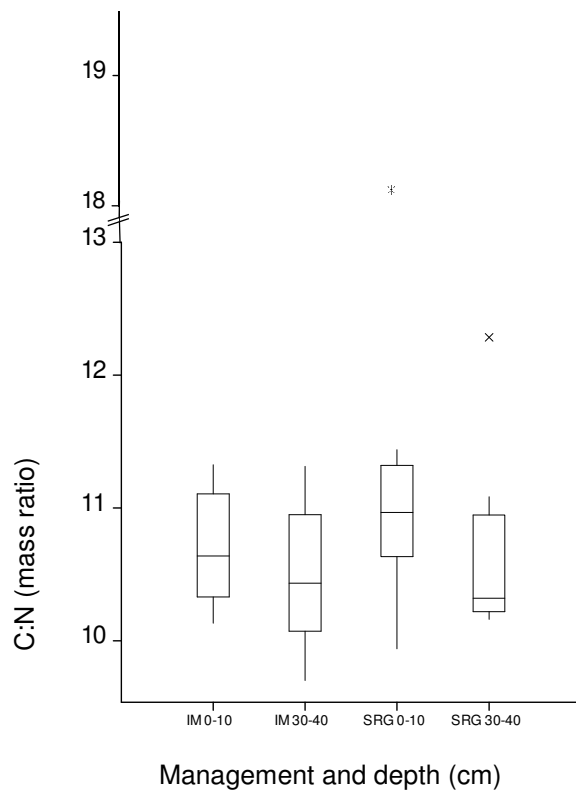


Figure 4.21 Box plot summarising the distribution of the 'plot means' for C:N (mass ratio), showing median, inter-quartile range, and range for samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm). Values lying > 1.5 times the inter-quartile range outside of the quartiles are shown as outliers

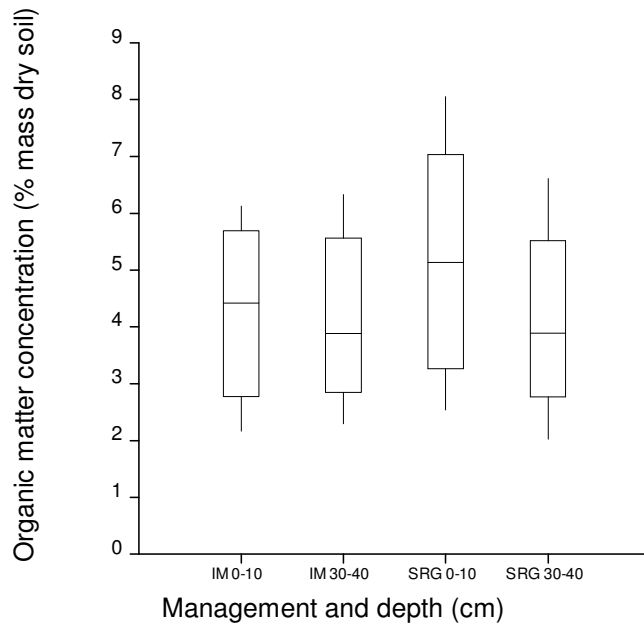


Figure 4.22 Box plot summarising the distribution of the 'plot means' for soil organic matter concentration showing median, inter-quartile range, and range for samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm).

4.3.3 Soil pH

All the soils sampled were slightly acidic, with the 'plot means' for pH ranging from 4.9 to 6.6 (Figure 4.23). Soil pH tended to be lower in the SRG plots, excluding an outlier, compared to the IM plots in the 0-10 cm depth range, in both the spring (Figure 4.23) and summer (Figure 4.24). In the 30-40 cm depth range the mean pH was greater in the SRG plots (6.21), than the IM plots (5.98). Thus the difference in pH between the two depth ranges in the SRG plots was greater than in the IM plots.

An ANOVA conducted on the 'plot means' for soil pH in spring 2010 and 2011 (Figure 4.23), showed a significant difference in mean soil pH between the IM and SRG plots ($F(1,3)=59.8$, $P=0.004$), with a mean ($n=16$) pH across both depths of 5.93 for the IM plots and 6.04 for the SRG plots (standard error of the difference of the means, $s.e.d = 0.014$). There was also a significant interaction effect on measured soil pH between the management type and the depth of soil sampling, ($F(1,6)=14.3$, $P=0.009$)

The mean ($n=8$) soil pH, in the 0-10 cm depth range in summer 2010/2011 (Figure 4.24), was higher in the IM plots than the SRG plots, with means of 6.08 and 5.75

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respectively (s.e.d. means=0.128), but an ANOVA showed this difference to be non-significant ($F(1,3)=6.61$, $P=0.082$).

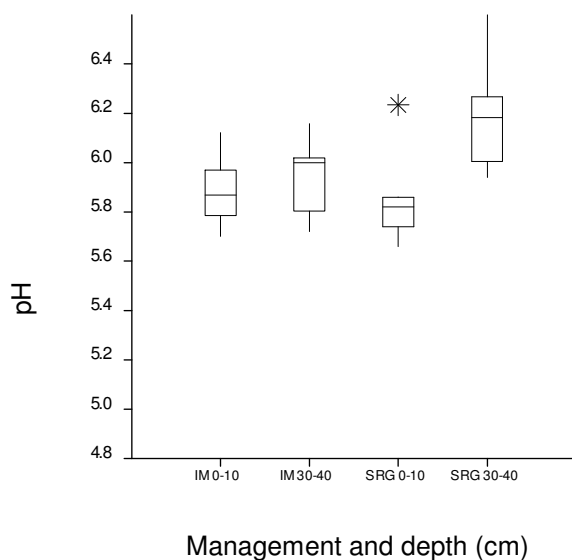


Figure 4.23 Box plot summarising the distribution of the 'plot means' for soil pH, showing median, inter-quartile range, and range, for samples collected in spring 2010 and 2011. Data grouped according to plot management (IM/SRG) and soil depth sampled (0-10 cm and 30-40 cm). Values lying >1.5 times the inter-quartile range outside of the quartiles are shown as outliers

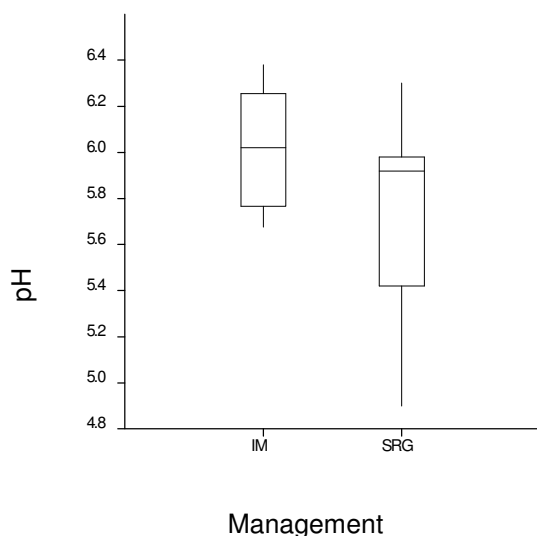


Figure 4.24 Box plot summarising the distribution of the 'plot means' for soil pH, showing median, inter-quartile range, and range of samples collected in summer 2010 and 2011 from 0-10 cm depth. Data grouped according to plot management (IM/SRG)

4.4 Plant species survey

This section presents the % cover from different plant groups for each SRG plot in 2010 and 2011. Plants are grouped according to whether they were present in the seed mix sown on establishment of the SRG (sown) or not (non-sown), and subsequently into grasses, leguminous, forbs (L-forbs) and non-leguminous forbs (NL-forbs). Also presented are the species diversity and richness and the % of seed mix species that successfully established for each SRG plot in 2010 and 2011. Finally The mean and range of Ellenberg Indicator (EI) values for nitrogen (EI-N), moisture (EI-moisture), light (EI-light) and pH (EI-pH) are presented for the species identified in each SRG plot.

4.4.1 Analysis of plant species data

Plant species data were analysed to identify the success of sown species in establishing at each SRG plot and to compare the botanical diversity between the SRG plots, as well as how diversity changed over the two years of sampling (2010 and 2011). Mean ($n=5$) % cover for each plot was calculated as the mean % cover for each category of plant over the five quadrats in each plot. Ellenberg indicators (EIs) for the established species in each plot were analysed as an indication of environmental conditions in the SRG plots. The range and mean of the EIs for N, light, moisture and pH for the species in each plot is presented. The mean EI for each plot was calculated by taking the mean of all the species identified in the five quadrats sampled in each plot. The EI values were not weighted according to abundance, as many authors have found this gives no improvement in correlation between EI indicator values and the measured value of a variable (Ertsen et al., 1998; Diekmann, 2003; Käfer and Witte 2004; Stevens et al., 2012). The Shannon diversity index (H) was calculated for each plot, using the mean % cover to determine the P_i for each species (Equation 11).

$$H = \sum_{i=1}^S -(P_i \times \ln P_i) \quad (11)$$

Where: P_i = the fraction of the entire plant population made up by species i

4.4.2 Percentage cover data

In all the SRG plots a greater mean % cover was provided by grasses compared to forbs and, with the exception of S3 SRG in 2010, the plant group providing the largest proportion of cover was the grasses not present in the original seed mix (non-sown grasses).

At Site S3 SRG there was 6.8% bare ground in 2010, whilst in 2011 there was no bare ground (Figure 4.25). The mean % cover provided by forbs increased from 8% cover (all from L-forbs) in 2010 to 27.2 % (22.6% from L-forbs) in 2011. The mean % cover from non-sown grasses stayed the same across the two years at 40.2%, whilst sown grasses showed a slight decrease

At site S5 sampling was only carried out in 2010, the plot S5 IM was under grassland, here *Lolium perenne* was the single dominant species, providing more than 99% of the cover. In contrast the mean % cover from forbs in plot S5 SRG was 45.8 % (Figure 4.26), the proportion of cover provided by sown and non-sown forbs was very similar at 21.5 and 24.3% cover respectively, the majority of the cover from non-sown forbs was provided by legumes, which provided 22.6% cover. The mean % cover from non-sown grasses was more than twice that of sown grasses at 38 and 16.2% cover respectively.

At plot S8 SRG, the percentage cover provided by each of the plant groups was very similar in both years (Figure 4.27). There was a slight increase in total forb cover, predominantly from non-sown forbs and a small decrease in sown grass cover. In both years over 80% of cover was provided by grasses, with 60% cover or more from unsown grasses. The majority of the cover from forbs was from sown legumes, which provided 15 and 10.2% cover in 2010 and 2011 respectively.

At site S9 SRG, the percentage cover from all the plant groups was also similar in both 2010 and 2011 (Figure 4.28), with only the sown grasses showing a substantial change in mean % cover, increasing from 6.21 to 13.8% cover. At both plots S8 and S9 SRG the mean % cover provided by sown forbs was more than four times that provide by non sown forbs in both years. In 2010 equal cover was provided by L

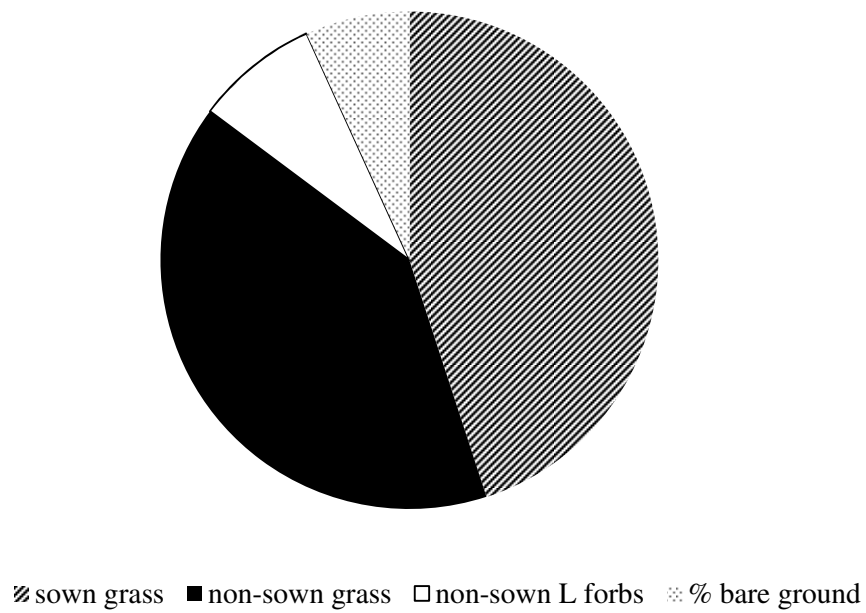
forbs and NL-forbs, whilst in 2010 L-forbs provided a total of 22.2% cover whilst NL-forbs species provided 19% cover.

4.4.1 Species diversity and richness

The SRG plot with the greatest diversity of all the Scottish plots was S9 SRG in both 2010 and 2011 (Table 4.3). The species richness was also greatest in plot S9 SRG in both years; in 2011 the species richness in the S9 SRG plot was more than 1.9 times greater than for the S3 and S8 SRG plots. In 2010 the lowest diversity was recorded in the SRG plot at site S3, whilst in 2011 the lowest diversity was found at site S8. The only plot at which an increase in diversity was observed between 2010 and 2011 was S3 SRG, where SDI increased by 0.62. Plots S8 SRG and S9 SRG showed a decrease in the SDI of 0.13 and 0.26 respectively between 2010 and 2011. In 2011 all three of the SRG plots samples showed an increase in species richness from the previous year.

The SRG plot in which the lowest percentage of the species from the seed mix were found to have established was S8 SRG; in 2010 three of the 13 seed mix species (23.1%) were observed across the five quadrats, in 2011 four of the seed mix species (30.8%) were observed. Less than 50% of the seed mix species were found to be established at plot S5 SRG, with seven of the 18 sown species (38.9%) observed in the plot in 2010. In 2010 the greatest % of seed mix species established was in plot S9 SRG with eight of the 13 sown species (61.5%) identified across the five quadrats, this figure was the same for 2011. Of the six species sown in the SRG at site S3, three (50%) were identified in 2010 and four (66.7%) were identified in 2011.

a)



b)

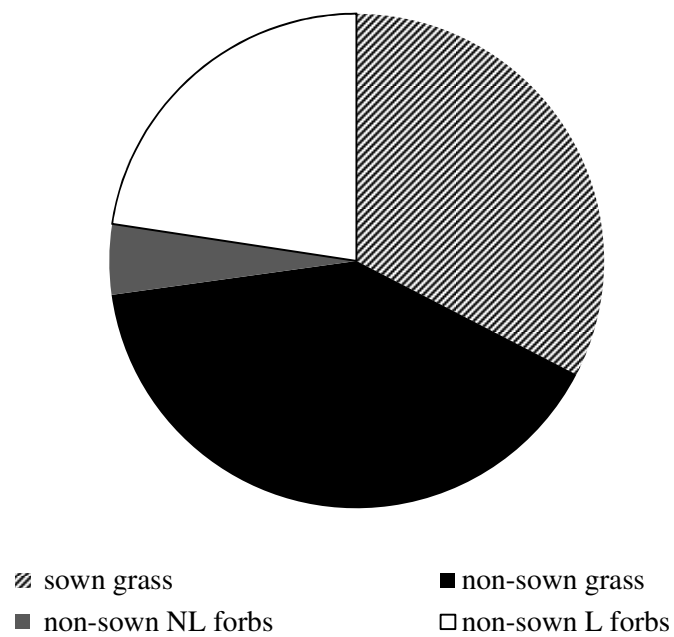


Figure 4.25 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown), non-sown L (L) and non-leguminous (NL) forbs and bare ground at plot S3 SRG in a) 2010 and b) 2011

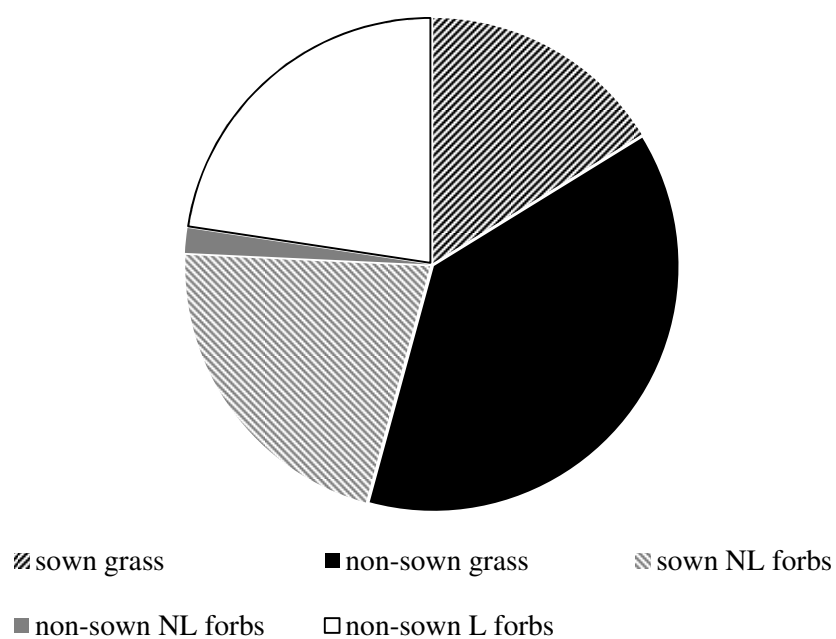
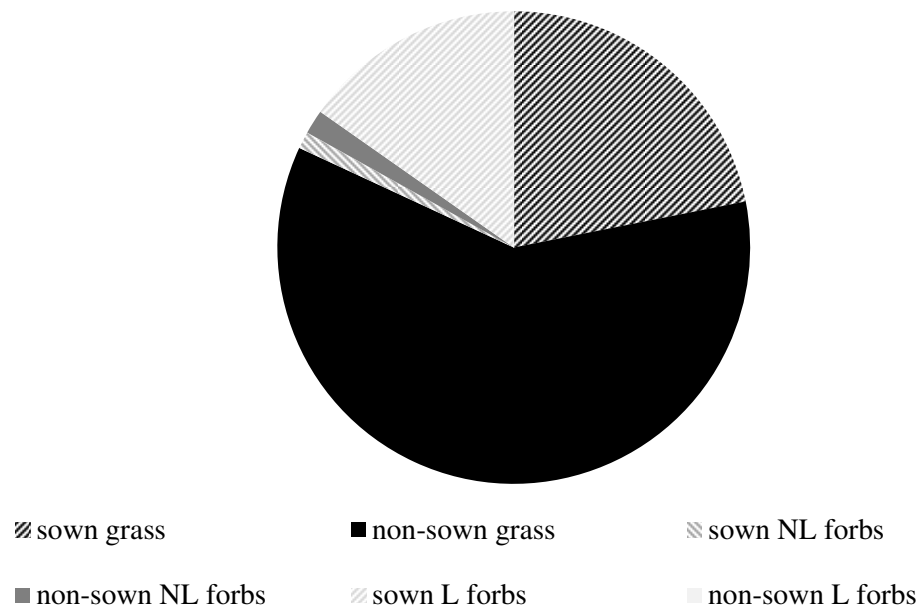


Figure 4.26 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown) and leguminous (L) and non-leguminous (NL) forbs that were either sown or non-sown at plot S5 SRG in 2010.

a)



b)

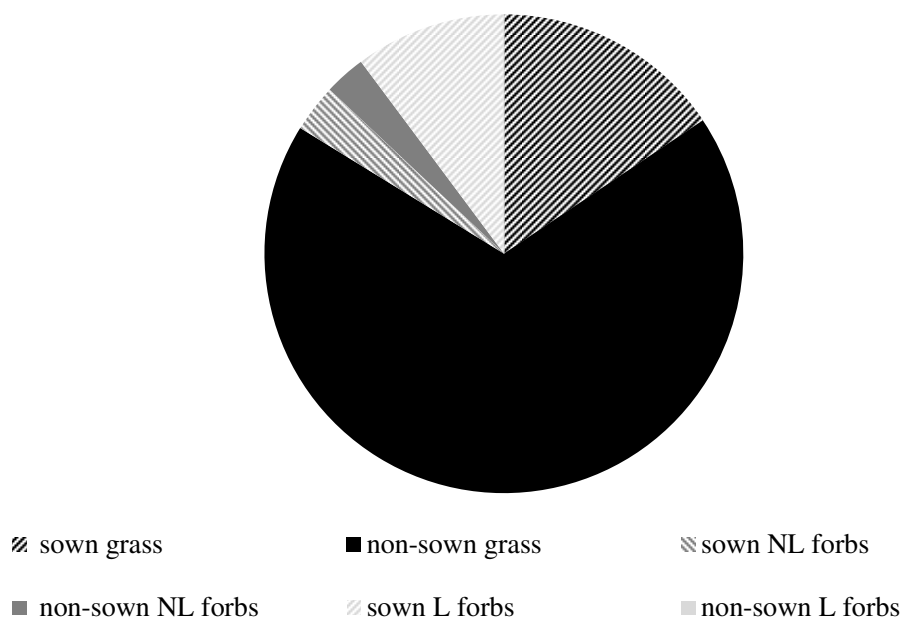
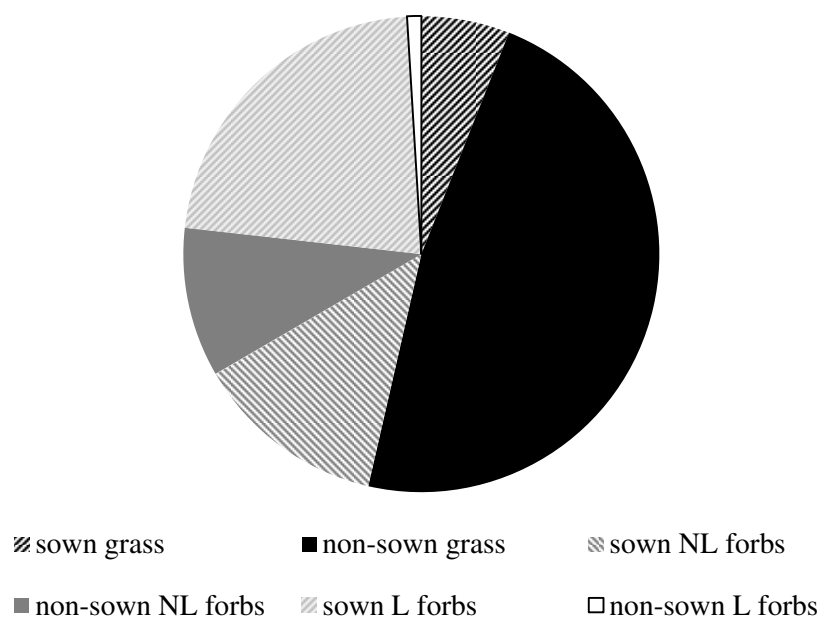


Figure 4.27 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown) and leguminous (L) and non-leguminous (NL) forbs that were either non-sown or unsown at plot S8 SRG in a) 2010 and b) 2011.

a)



b)

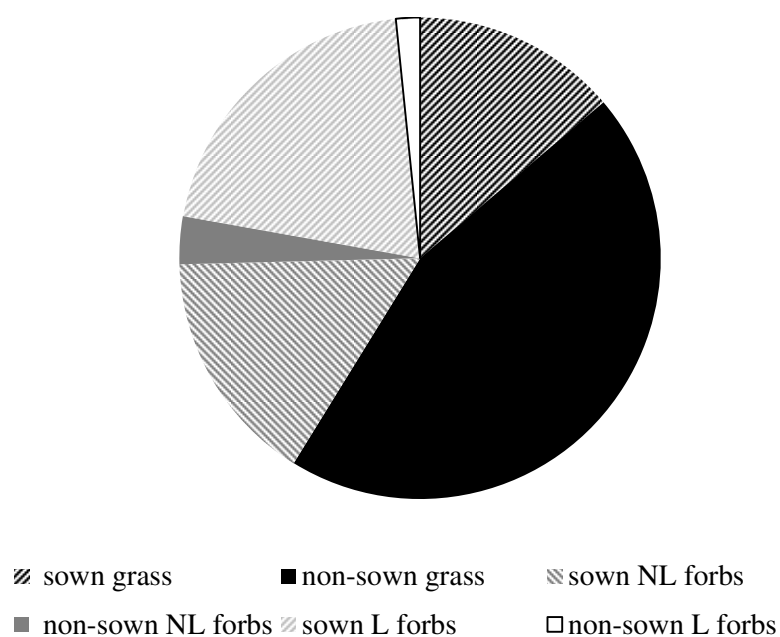


Figure 4.28 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown) and leguminous (L) and non-leguminous (NL) forbs that were either sown or non-sown at plot S9 SRG in a) 2010 and b) 2011.

Table 4.3 Summarizing the diversity, species richness and the percentage of all species sown in the seed mix that were established in each Scottish SRG plot in 2010 and 2011. Results are from identification of all vascular plant species within five 1 m x 1 m quadrats in each plot, quadrats were located using random numbers.

Plot	Shannon diversity index		% of seed mix species which have established		Total species richness	
	2010	2011	2010	2011	2010	2011
S3 SRG	1.33	1.95	50.0	66.7	8	11
S5 SRG	1.66	NA	38.9	NA	11	NA
S8 SRG	1.82	1.69	23.1	30.8	9	11
S9 SRG	2.33	2.07	61.5	61.5	15	21

4.4.1 Ellenberg indicator values of established species

The widest range in EI-N was observed in S8 SRG in 2011, which contained plants with EI-N ranging from 2-9. The modal range of EI-Ns for all the sites was 2-7 (Table 4.4). Only plot S9 SRG maintained the same EI-N range between 2010 and 2011. The range in EI-N values for N widened by two for plot S8 SRG and narrowed by 1 for plot S3 SRG between 2010 and 2011. The greatest mean EI-N for the Scottish SRG plots was 5.25, observed in 2010 in plot S3 SRG. The mean EI-N was < 5 for all the remaining Scottish plots in 2010. Plot S9 SRG showed a decrease in mean EI-N from 4.71 in 2010 to 4.47 in 2011, this was the lowest mean EI-N value for any site in either year. Plot S8 SRG showed the greatest increase in mean EI-N from 2010 to 2011

The modal range in EI-light was 6-8, no deviation from this modal range greater than 1 EI unit was recorded. The greatest change in mean EI-light between 2010 and 2011 was at plot S3 SRG where the mean EI increased by 0.77 units from 6.35 to 7.12. The mean EI-light for plot S9 SRG increased by 0.35 EI units from 6.69 in 2010 to 7.14 in 2011. The only plot to show a decrease in mean EI-light between 2010 and 2011 was S8 SRG, with a decrease of 0.37 EI units from 6.98 to 6.61.

The modal range in EI-moisture was 4-6, this was the range for all the plots in 2011 and plot S5 SRG in 2010. The narrowest ranges in EI-moisture were found at plots S3 SRG and S8 SRG in 2010, EI-moisture ranges of 5-6 and 4-5 respectively. Plot S3 SRG showed an increase in mean EI-moisture between 2010 and 2011 with an increase of 0.51 EI units from 4.47 to 4.98, plots S8 SRG and S9 SRG showed a decrease in mean EI-moisture between 2010 and 2011.

The EI-pH for all plots except S5 SRG ranged from 2-7 in both 2010 and 2011. The range of EI-pH values in plot S5 SRG was much narrower (4-6). Plots S3 SRG and S9 SRG both showed an increase in mean EI-pH between 2010 and 2011 of 0.59 and 0.29 EI units respectively, plot S8 SRG showed a decrease in mean EI-pH of 0.42 EI units over the two years

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Table 4.4 Mean and range of EI values for N, light, moisture and pH of all plants identified within the five 1m x 1 m quadrats in each Scottish SRG plot in 2010 and 2011. Quadrats located using random numbers.

Plot	N - Range of EI values		N - Mean of EI values		Light - Range of EI values		Light - Mean of EI values		Moisture - Range of EI values		Moisture - Mean of EI values		pH - Range of EI values		pH - Mean of EI values	
	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
S3 SRG	3-7	3-6	5.25	4.91	6-8	6-9	6.35	7.12	5-6	4-6	4.47	4.98	2-7	2-7	5.21	5.8
S5 SRG	3-9	NA	4.91	NA	7-8	NA	7.36	NA	4-6	NA	4.91	NA	4-6	NA	6.18	NA
S8 SRG	2-7	2-9	4.63	5.18	6-8	6-8	6.98	6.61	4-5	4-6	5.01	4.73	2-7	2-7	5.86	5.44
S9 SRG	2-7	2-7	4.71	4.47	6-7	6-9	6.79	7.14	4-7	4-6	5.14	4.95	2-7	2-7	5.57	5.86

4.5 Soil WFPS and temperature

4.5.1 Analysis of soil physical data

The BD, WFPS and soil temperature measured on each sampling occasion for paired IM and SRG plots were analysed using a student's T-test to compare the measurements from the two plots. Prior to carrying out a test the data were checked for equality of variances (Section 3.12). The differences in the mean ($n=5$) BD at paired IM and SRG plots were plotted against the age of the SRG plot, the Pearson's product-moment correlation coefficient (R^2) was then calculated for the two variables.

4.5.2 Summary of parameters used to calculate soil WFPS

Soil BD and organic matter content (Sections 3.6.2 and 3.6.4) were measured in March 2010 and used along with gravimetric water, measured on each gas sampling occasion, to calculate WFPS (Section 3.6.5). The mean BD ranged from 0.991 ($\pm 0.0672 \text{ g cm}^{-3}$) to 1.61 ($\pm 0.138 \text{ g cm}^{-3}$) across all plots (Figure 4.29).

There was a trend for an increasing difference between the mean BD of paired IM and SRG plots with increasing age of the SRG (Figure 4.30) in both the 0-10 cm ($R^2=0.98$) and the 30-40 cm ($R^2=0.83$) depth ranges. At the site pair with most recently established SRG (S3), soil BD was significantly lower in the IM plot than the SRG plot in the 30-40 cm depth range ($T(5)=3.49$, $P=0.025$). At the site pair with an intermediate age SRG (S5) soil mean BDs were fairly similar in the IM and SRG plots. The mean soil BD in the 0-10 cm depth range at site S8 was significantly greater in the IM plot compared to the SRG plot ($T(8)=2.83$, $P=0.0222$). The soil BD in the longest established SRG plot, Site S9, was significantly lower than in the paired IM plot at both the 0-10 and 30-40 cm depth ranges ($T(8)=3.59$, $P=0.00711$ and $T(5)=4.51$, $P=0.00637$ respectively)

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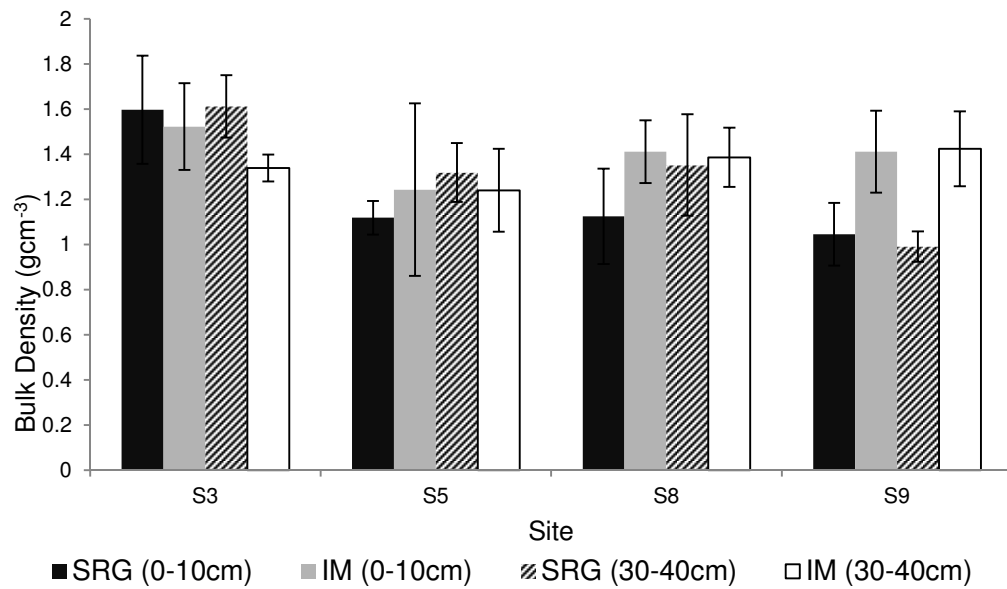


Figure 4.29 Mean ($n=5$) soil BD for two depth ranges at each plot (IM and SRG) for the four Scottish sites (S3, S5, S8 and S9). Error bars show ± 1 SD.

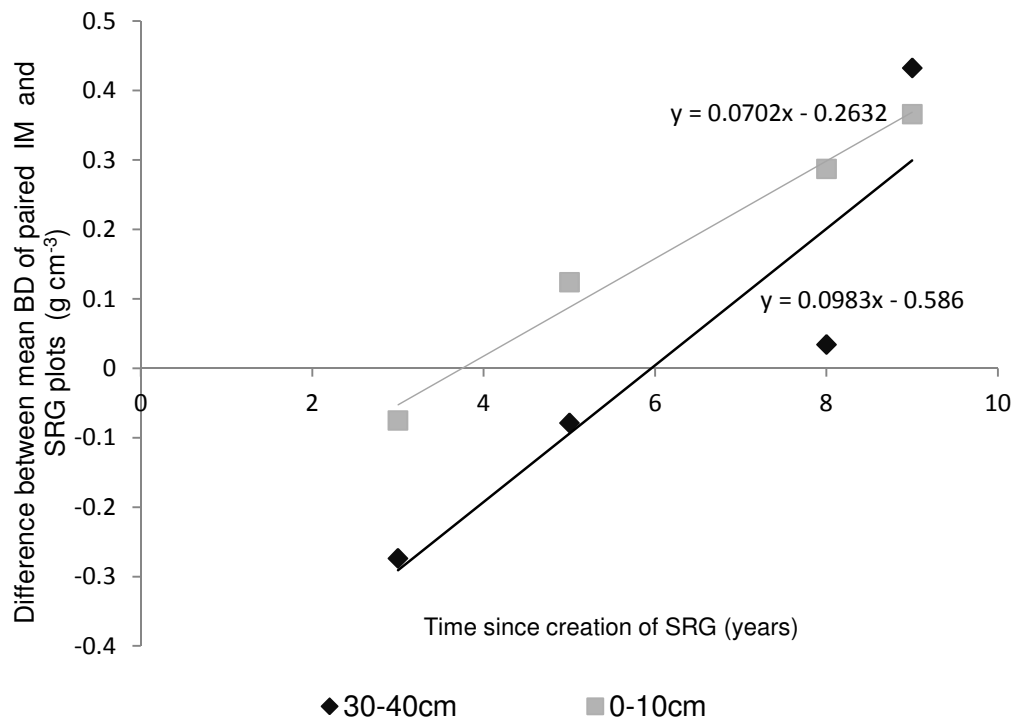


Figure 4.30 Difference in mean BD between paired IM and SRG plots in spring 2010 (IM-SRG), plotted against time since creation (age) of the respective SRG plot. Trend lines fitted using Microsoft Excel. Regression equations are shown adjacent to each trend line.

4.5.3 Summary of WFPS results alongside daily rainfall data

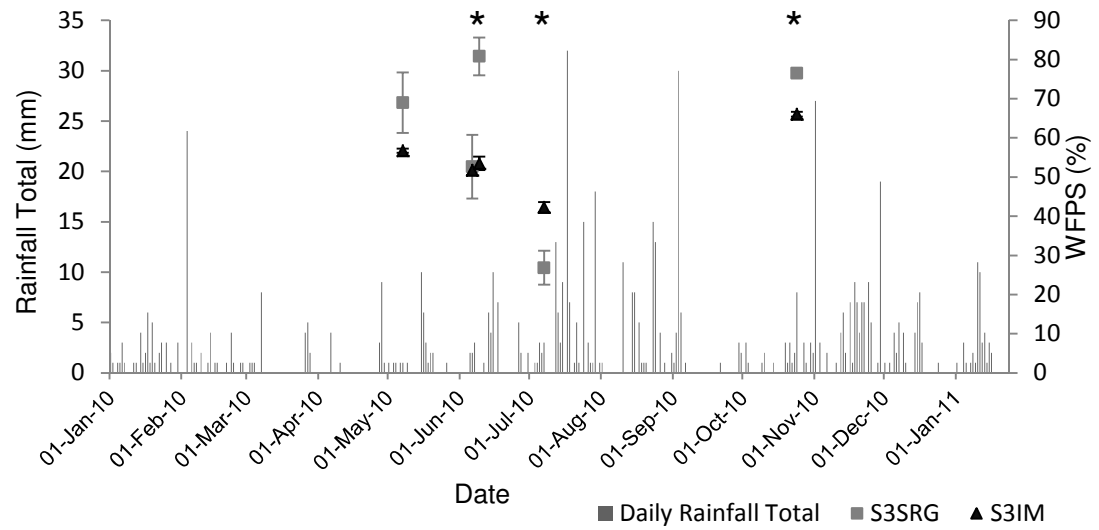
The mean (n=3) soil WFPS in the plots at the time of N₂O gas flux measurement varied between 25.2 and 110% across all plots (Figure 4.31). On some sampling occasions there was large within plot variation, leading to high standard deviation from the mean, but mean WFPS was often similar between paired IM and SRG plots. Many authors have found substantial N₂O emissions from denitrification above 60% WFPS (Dobbie et al. 1999; Bateman and Baggs 2005; Russer et al. 2006), although N₂O emissions can still occur below this level. Table 4.5 summarises the number of occasions on which soil WFPS was > 60% for each plot.

Table 4.5 Summarising the number of occasions on which measured soil WFPS at the time of N₂O flux measurements was >60% for each plot at the four Scottish sites.

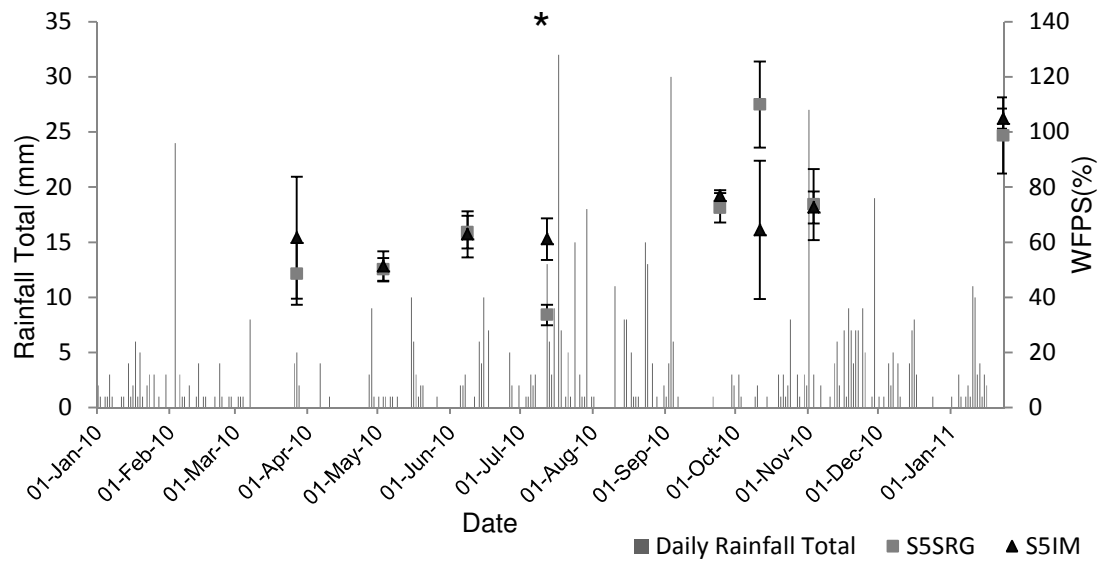
Site	Number of occasions WFPS >60% for each plot	
	IM	SRG
S3	1	3
S5	7	5
S8	3	1
S9	3	2

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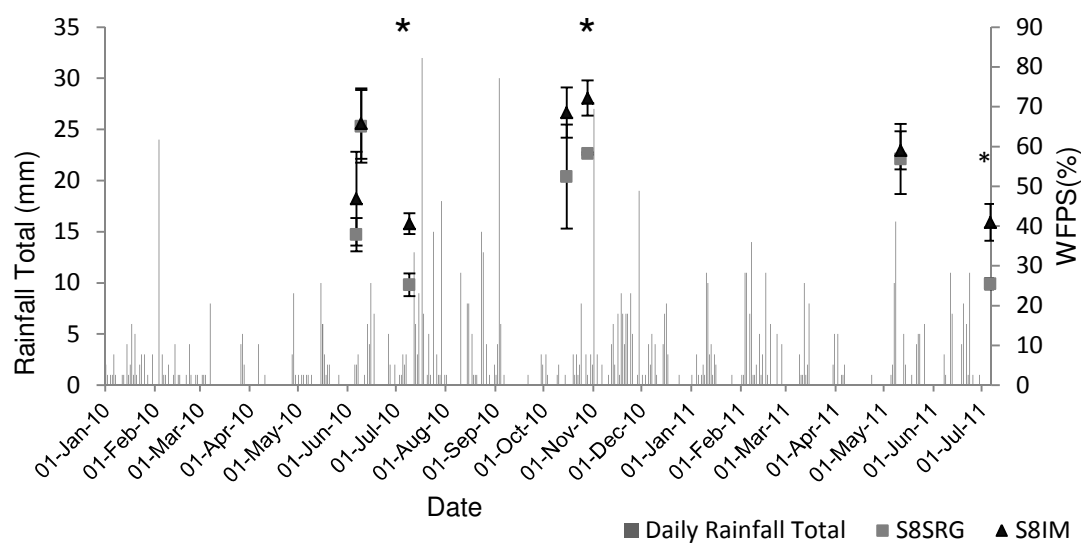
a) site S3



b) site S5



c) site S8



d) site S9

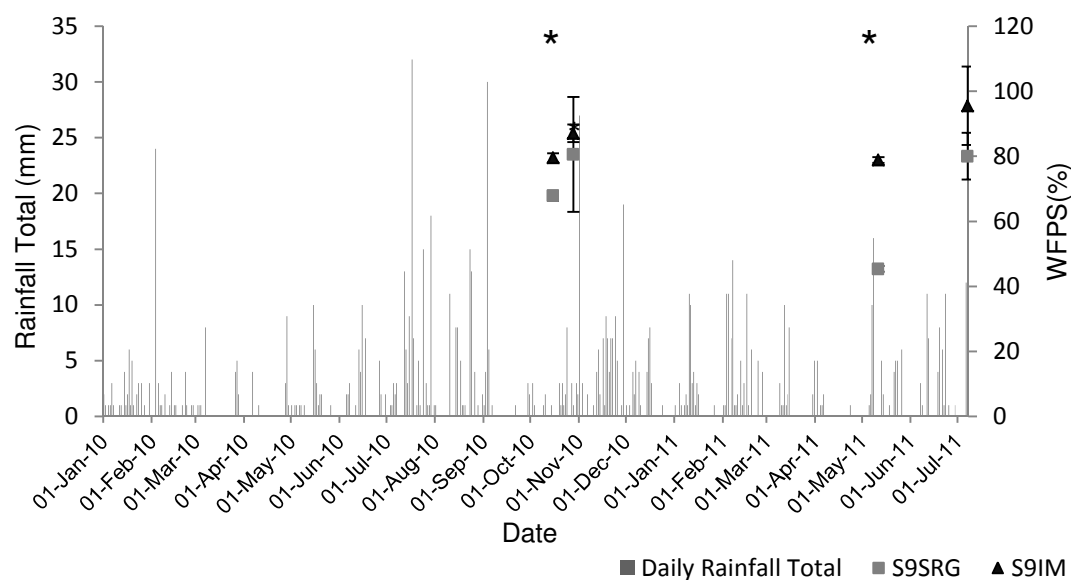


Figure 4.31 Daily rainfall totals measured at Greenlaw met. station with mean ($n=3$) soil WFPS on each gas sampling occasion for each plot pair IM/SRG at the four Scottish sites a)S3, b)S5, c)S8, d)S9, error bars show ± 1 SD. An asterisk indicates where the WFPS at the IM and SRG plots differ significantly ($P<0.05$).

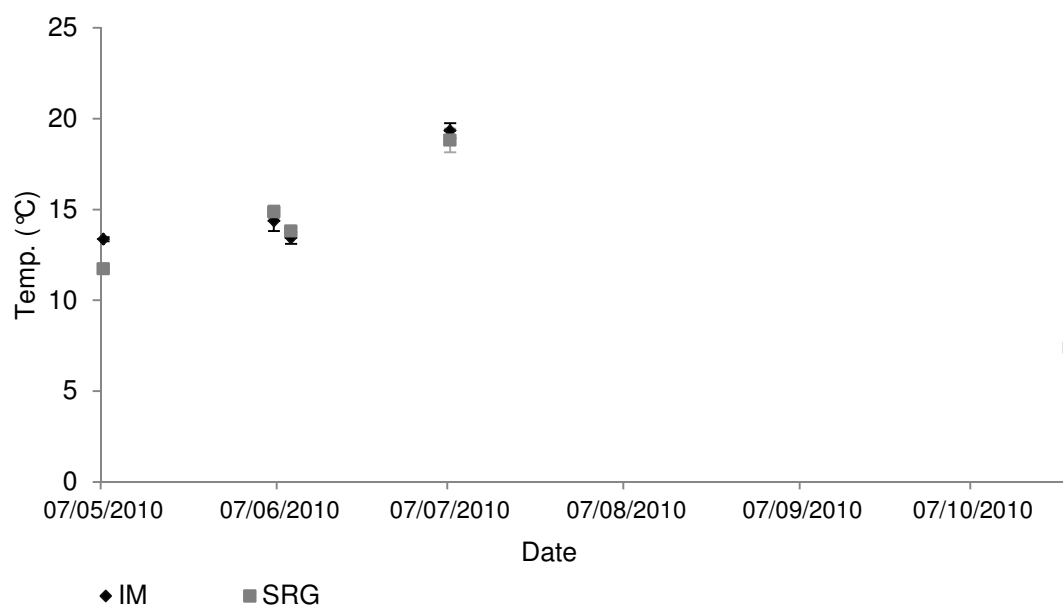
4.5.4 Soil temperature at time of gas sampling

The soil temperature was measured as it is a key driver effecting rates of N_2O emissions and required for the calculation of N_2O fluxes from gas concentration data (Equation 10). The mean soil temperatures measured across all four site pairs ranged

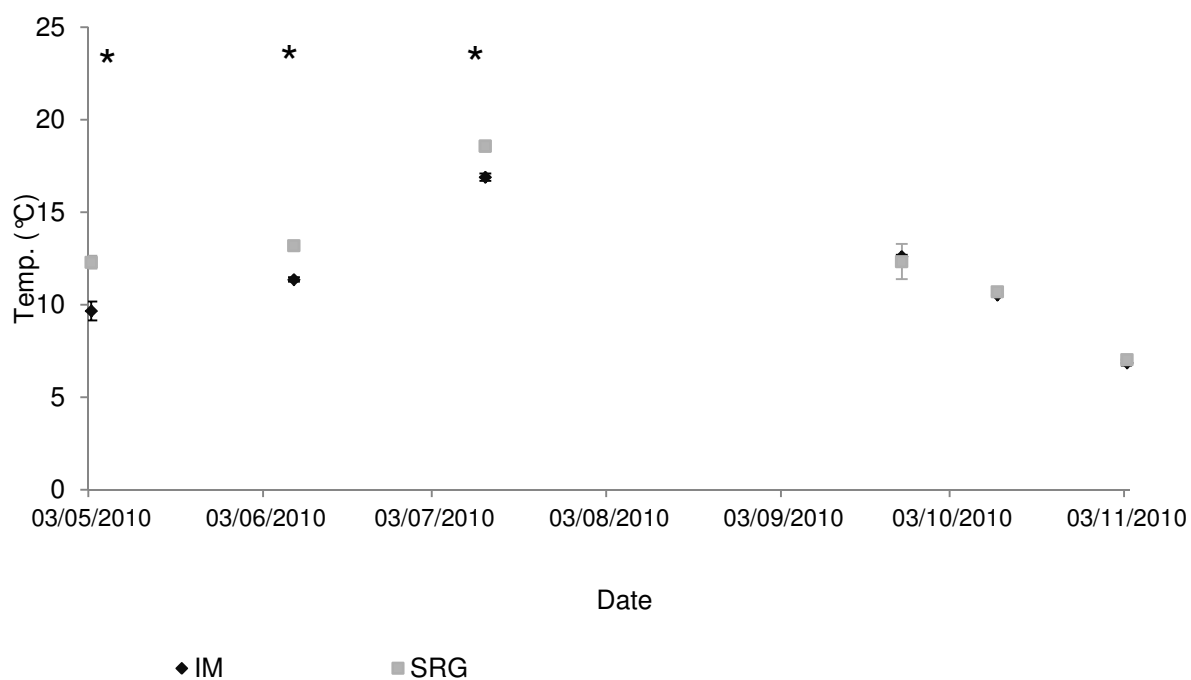
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between 5 and 20 °C (Figure 4.32) over the two year period. With the exception of site S5 and one occasion at site S8, the mean soil temperatures at paired IM and SRG plots differed by less than 1 °C (Figure 4.32). The similarity in soil temperatures at paired IM and SRG plots, suggests that differences in soil temperature should not have a significant effect on the gas fluxes measured at paired plots.

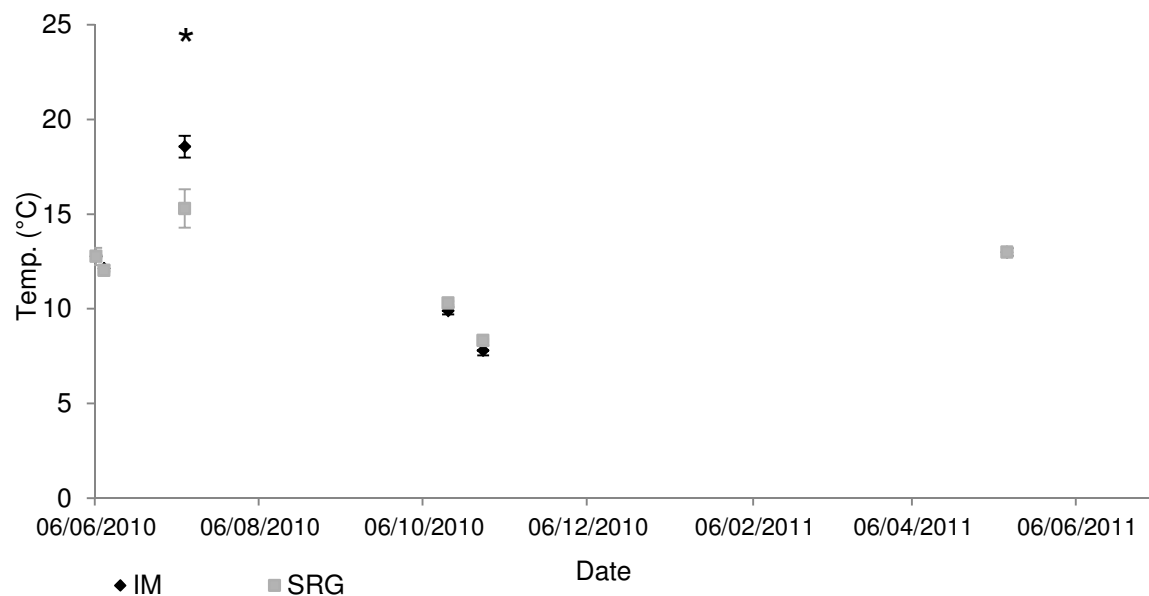
a)



b)



c)



d)



Figure 4.32 Mean ($n=3$) temperature of top 2cm of soil at time of gas sampling in IM and SRG plots at the four Scottish sites a) S3, b) S5, c) S8, d) S9. Error bars $\pm 1SD$. An asterisk indicates where paired temperatures differed significantly between paired IM and SRG plots.

4.6 N₂O flux measurements

4.6.1 Analysis of N₂O flux data

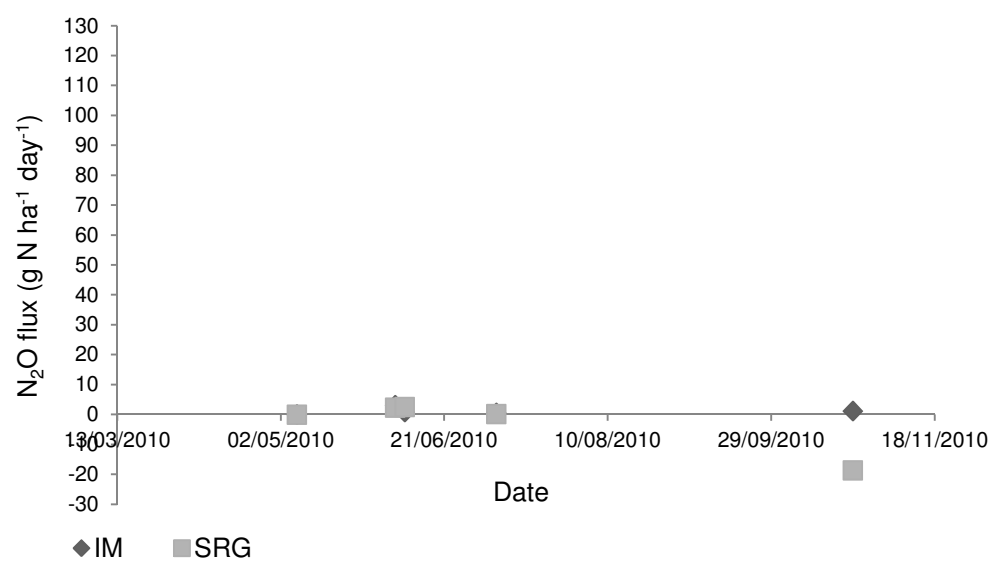
Data from N₂O flux measurements were analysed to identify any effect of management on soil N₂O emissions. The soil N₂O flux sampling occasions were not sufficiently frequent or evenly spaced in time to carry out a time series analysis, so a

separate two sample T-test was conducted in Microsoft excel on the N₂O flux values from the three chambers in each plot on each sampling occasion. Although

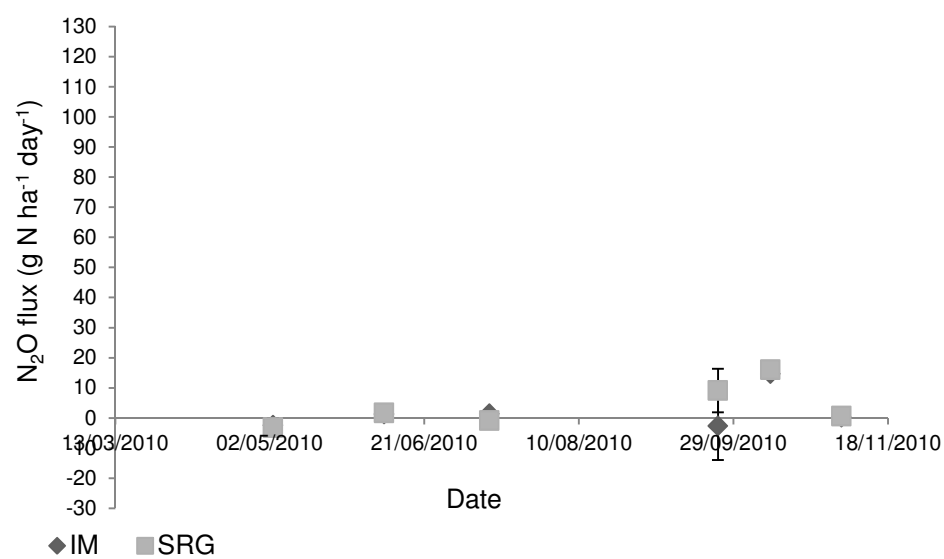
4.6.2 N₂O flux data

With the exception of one occasion at site S8 and S9, the mean N₂O fluxes measured were very low (below 20 g N ha⁻¹ day⁻¹) from both plots at each site, with occasional negative fluxes being recorded (Figure 4.33). There was a very slight increase in the background N₂O flux in September and October 2010 from both plots at site S5 (Figure 4.33b), but the only substantial peaks, > 20 g N ha⁻¹ day⁻¹, in mean N₂O flux were measured in the IM plots of site pairs S8 and S9 on 11 May 2011 (Figure 4.33c & d). On these occasions the standard deviation in the measurements from the three chambers in the IM plot was large, so the difference was found to be statistically insignificant for both site S8 ((T(2)=2.63, P=0.119) and S9 ((T(2)=2.55, P=0.125)).

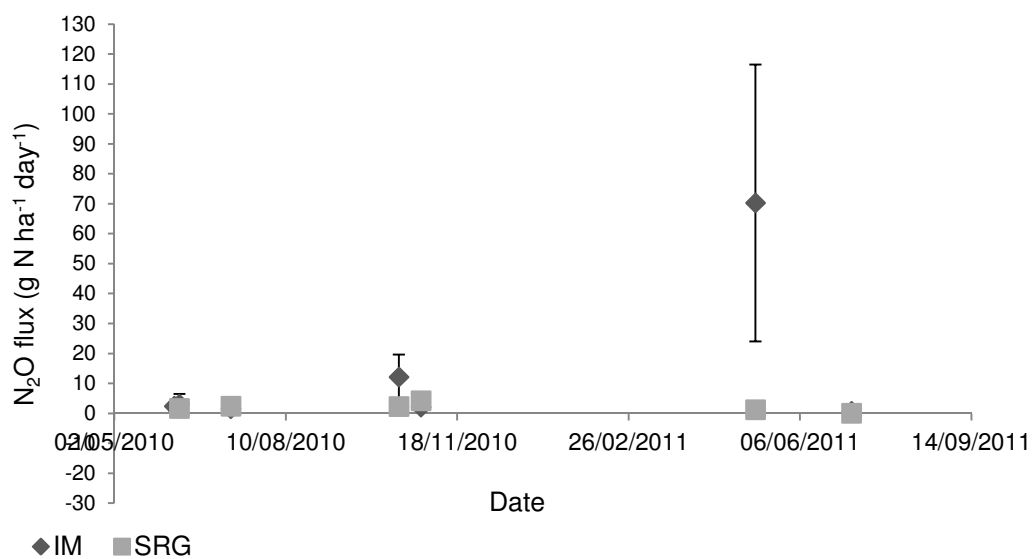
a) site S3



b) site S5



c) site S8



d) site S9

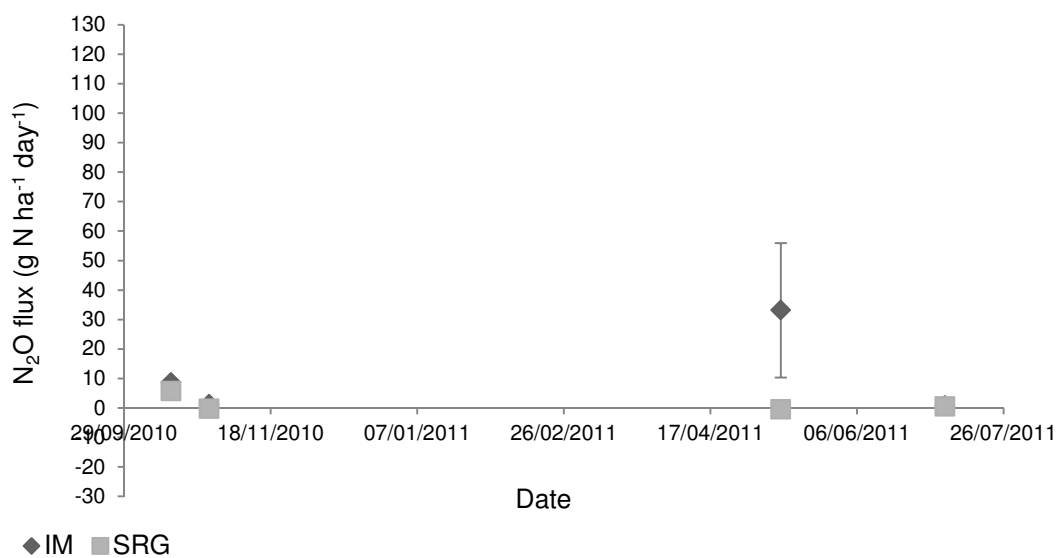


Figure 4.33 Mean (n=3) N₂O flux recorded at site a) S3, b) S5, c) S8 and d) S9. Error bars show ± 1 SD.

4.7 Discussion

4.7.1 The effect of cessation of intensive management on soil N, C and organic matter concentration

All the Scottish SRG plots in the study historically received inorganic fertiliser when under intensive management. It was hypothesised that following the cessation of intensive management such sites would be left depleted in total soil N (Knops and Tilman 2000), and that the limited availability of organic N for mineralisation and nitrification could result in the available N contents being lower than the paired IM plots, which were still receiving inorganic fertiliser applications (Section 1.7). Rates of N mineralisation were not directly measured in this study due to insufficient time and funds. However mineral N values were compared between the site pairs and, despite the cessation of fertiliser applications, the available soil N at the SRG plots was not found to be significantly lower than at the paired IM plots. The inorganic N, applied in the form of NO_3^- and NH_4^+ when the SRG plots were under intensive management, would have been readily taken up by the crop plants and soil microbes and, in the case of highly labile nitrate, rapidly leached from soil. Hence the available soil N present in the SRG plots is unlikely to have originated directly from excess fertiliser but be derived from mineralisation of OM. Despite the lack of organic fertiliser application under IM management, organic N-rich compounds could have accumulated during intensive management from crop residue and soil microbes which utilised the mineral fertiliser N. The N concentration at the SRGs could also be enhanced by biological N fixation from established legumes. Atmospheric deposition could also contribute to the maintenance of high available soil N concentrations at the SRGs despite the cessation of fertiliser applications, (Janssens et al., 1998) although rates of both wet and dry N deposition at the study sites in Scotland are likely to be low, totalling $< 14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ at site S5, closest to Edinburgh and $< 10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ at sites S3, S8, and S9 (Centre for Ecology and Hydrology, 2012).

The N supply to the soils in the SRG plots in this study could be enhanced by high levels of soil P, which readily accumulates under intensive management. Some authors have found that high soil P encourages the dominance of leguminous plant

species (Bobbink, 1991; Davis, 1991; Israel, 1991; Mamolos et al., 1995), leading to high rates of biological N fixation. The results from the plant surveys conducted in the SRG plots show leguminous species provided between 10 and 23% of the total plant cover. The majority of the cover from forb species was from L-forbs, with NL-forbs being much less dominant. Janssens et al. (1998) suggested that high soil P may also encourage N mineralisation, by enabling high rates of microbial activity and encouraging plant growth and the production of high quality, readily mineralised plant matter. In addition, agricultural practices such as tillage are known to impact on soil structure by breaking up aggregates and reducing the physical protection of soil N (Mikha and Rice, 2004). The effect of tillage on soil structure can be long lasting and it is likely that it continues to encourage N mineralisation for many years following the cessation of intensive management (Knops and Tilman, 2000)

The hypothesis that high mineral N concentrations are maintained in the SRG plots through rapid mineralisation of organic N, is supported by the comparatively low concentrations of total N. No significant difference was found between the soil total N concentrations at the IM and SRG sites and soil total N concentrations in all plots were lower than those reported at long-established semi-natural grasslands. Gough and Marrs (1990) found a mean soil total N content of 0.55% mass at long established semi natural grasslands in the UK, compared to 0.295% mass at adjacent IM sites. Janssens et al. (1998) studied permanent, species-rich grassland in Western Europe and found soil total N contents ranging from 0.3 to 0.87% dry mass. The low total N concentrations in this study, agree with the findings of other authors and support the hypothesis that changes in soil total N following cessation of intensive agriculture occur very gradually over many decades (Knops and Tilman, 2000). The similar low total N and high available N contents at both the SRG and IM plots in this study suggest that N cycling under both management types is dominated by mineralisation over immobilisation. This could limit ES provision, by increasing the likelihood of nutrient leaching (Hatch et al., 2002), thus limiting the potential for water quality regulating services. High available N can also lead to increased denitrification and N₂O emissions, hence a poor provision of climate regulating services from newly created SRG compared to long established SRGs (Dobbie et al.,

1999). Finally, a relatively high concentration of available N in the SRG plots, could limit their potential to support high biodiversity grassland (Critchley et al., 2002).

The ratio of available $\text{NH}_4^+\text{-N}:\text{NO}_3^-\text{-N}$ appeared to be slightly greater in the SRG plots compared to the IM plots in the summer soil samples. This difference was found to be insignificant; however it could indicate the need to study a greater number of sites to determine whether increasing the size of the dataset reveals a consistent and significant effect of management on the $\text{NH}_4^+\text{-N}:\text{NO}_3^-\text{-N}$ ratio. A higher ratio of available $\text{NH}_4^+\text{-N}:\text{NO}_3^-\text{-N}$ could reduce the risk of nitrate leaching from soils (Jarvis and Barraclough, 1991). However it may increase the risk of NH_3 volatilization (Bouwman et al., 2002).

The fact that the C:N ratio is very similar across all plots, mostly between 10.5 and 11.4, concurs with the findings of other studies, which have suggested that a highly conserved C:N elemental ratio exists across terrestrial ecosystems. Cleveland and Liptzin (2007) found an average bulk soil C:N ratio of 13.8 ± 0.4 for grasslands not exposed to tillage, fertiliser or intensive agriculture. Slight increases in the C:N ratio have been found to occur with increasing age of grasslands, and are an indication of increasing N limitation, which can result in decreasing net N mineralisation (Accoe et al., 2004). This study found no significant difference in soil C:N ratio between the SRG and IM plots, the mean C:N ratio in all plots was lower than any found by Cleveland and Liptzin (2007) in long established grasslands. The findings suggest that a longer period is needed for the C:N ratio in the SRG plots to increase to values similar to those found by Cleveland and Liptzin (2007) following cessation of intensive agriculture. The low soil C:N ratio is further evidence, along with the relatively high available soil N compared to total N, that both the SRG and IM soils experience high net mineralisation, which could encourage N leaching and gaseous N_2O emissions. However care should be taken when interpreting C:N ratio results, since other authors have shown that measuring only the total soil C:N ratio can mean significant changes in the C:N ratios of the smaller more active soil pools, such as rapidly cycled organic compounds produced by live microbes, are missed (Piñeiro et al., 2006) these active pools can have the greatest effect on soil N cycling.

Despite the very similar concentrations of available N, and total N and C:N ratios in the soils at the IM and SRG plots, the organic matter concentration, whilst not significantly different between plots, tended to be greater in the 0-10 cm depth range of the SRG plots, compared to the IM plots. An accumulation of organic matter following cessation of fertiliser application could signal the start of a change in chemical properties of the soil under SRG management. It has been reported that the first indication of organic matter accumulation and increase in storage of C and N following conversion to grassland, is an increased concentration of high density macro-organic matter, with particles of $>150\text{ }\mu\text{m}$ in diameter (Accoe et al., 2004). Determination of the quality and distribution of the organic matter among size and density fractions under the two management types in this study, would further determine the likely impact of conversion to SRG on N cycling. Overall the soil analysis results for the IM and SRG plots suggest that, following conversion to SRG, the provision of N cycling services is unlikely to be significantly enhanced for at least 10 years after conversion, as net N immobilisation and mineralisation are likely to be similar in the paired IM and SRG plots. The mostly recently created SRG in this study was three years old in 2010, whilst 2011 was the 10th year since the oldest SRG site (S9) was established. There is no evidence of any effect of the age of the SRG on any of the measured soil chemical properties and how they compare to the paired IM plot. This lack of an age-related effect, suggests it will take more than 10 years for a significant change in soil chemical properties to occur following conversion from IM to SRG.

4.7.2 The effect of the cessation of intensive management on soil P

Soil P concentrations can take many years to decline following cessation of fertiliser application (Dodd et al., 2012). It was predicted that the soil total and available P would be similar for both the IM and the SRG plots in this study, as fertiliser application to even the oldest of the SRG sites only ceased 10 years prior to sampling. The results support this hypothesis, with no significant difference in either total or available soil P being found between the IM and SRG plots.

It is difficult to directly compare the available soil P measured in this study with the concentrations recorded by others in agricultural and semi-natural sites due to

differences in the soil extraction methods used. Using the Olsen P extraction, Gilbert et al. (2009) found an available P concentration of between 11 and 40 mg kg⁻¹ in 65% of arable soils, whilst the available P in species-rich communities tended to be lower (between 3 and 10 mg kg⁻¹). Using the acetic acid extraction method (as used in this study) Gilbert et al. (2009) found that the available P concentrations determined in soils across a range of species-rich and species-poor communities were of a similar magnitude to, but less strongly correlated to species richness than the concentrations measured with the Olsen P extraction method. The role of P in limiting biodiversity provision from semi-natural grasslands has been well documented (Critchley et al., 2002; Walker et al., 2004). As well as reducing the potential of biodiversity to be enhanced in SRGs, the fact that available P in the SRG plots is similar to adjacent IM plots, will likely result in the establishment of plant species of lower conservation status, that are commonly found around the margins of intensively managed sites (Linusson et al., 1998). The plant species data collected, identify species in the SRG plots with a wide range of EI-N values, ranging from species usually found at low fertility sites such as *Lotus corniculatus* (EI-N of 2) to those found on extremely fertile sites such as *Rumex obtusifolius* (EI-N of 9). Overall the mean EI-N values for the SRG plots are greater than reported for other high-diversity European grasslands containing species of high conservation value, and are more similar to the mean EI-N values of low-quality, degraded grasslands, (Section 4.7.5). Furthermore, the species diversities recorded in the SRG plots were lower than those reported for high biodiversity grasslands (Section 4.7.6). Therefore the plant species data support the hypothesis that high soil fertility is limiting the potential for the SRG plots to support diverse communities containing species of high conservation value.

The finding that available soil P concentrations are similar and relatively high across both soil depths sampled in all plots, suggests the rates of P loss in leachate are likely to be similar from both management types. Many authors have reported positive correlations between soil test P and P loss in leachate (Hesketh and Brookes, 2000; McDowell et al., 2001a). In addition to soil chemical analysis, the analysis of leachate from the study sites would be useful in quantifying the rate of P loss which, whilst often correlated to soil test P, has been found to have a non-linear relationship

Chapter 4 Results and discussion from Scottish sites

with soil P concentration (Heckrath et al., 1995) and can be affected by other factors such as soil structure and hydrology (Djodjic et al., 2004; Maguire and Sims 2002). In order to directly measure the rates of soil P loss in solution, leachate collection was attempted from the sites but was unsuccessful due to insufficient volumes of water being collected. The available soil P data from this study however, suggest that any enhancement in water quality regulation (reduction in P leaching) or biodiversity provisioning (establishment of plant species of higher conservation value) services following conversion to the SRG, are likely to have been limited for the sites in this study.

There was also no significant difference in the soil total P concentration measured at the IM and SRG sites. The total P pool has the potential to gradually de-sorb from soil particles and become available to plants and susceptible to leaching loss (Koopmans et al., 2004; Vu et al., 2010). Thus, high residual soil P could continue to sustain available soil P concentrations and rates of P loss in leachate for many years (Meals et al., 2010). As a result, long term ES provision from the SRGs in this study could be limited by excess P. Substantial concentrations of insoluble P, or P that is tightly sorbed to soil particles (Figure, 2.1) can also enter ground water (Holman et al., 2008) and surface water through erosion (Withers and Jarvie, 2008), where it can be harmful due to long term accumulation and gradual mineralisation (Seitzinger and Sanders, 1997). Whilst soil P content remains high, any improvement in ES provision may be limited. Further work at the study sites to assess the extent of this limitation could include quantifying the risk of P loss through soil erosion, which is determined not only by total P concentration but also by soil structure and susceptibility to erosion (Borda et al., 2011).

As with the other soil chemical properties, the lack of any age related effect on soil P concentration in the SRG plots, suggests that it will take longer than 10 years following conversion to SRG before any significant change occurs in soil P concentration.

4.7.3 The effect of the cessation of intensive management on soil pH and the significance for ecosystem function

Soil pH is potentially important in regulating ES provision, having been found to have a strong influence on the type of plant community that establishes, although plant communities of high botanical value have been found across a wide range of soil pH values (Janssens et al., 1998; Critchley et al., 2002). Soil pH can also affect the soil microbial community composition (Baath and Anderson, 2003), and has been shown to be positively correlated with gross N mineralisation, gross nitrification, and soil NO_3^- -N concentration and negatively correlated with NH_4^+ -N concentration (Cookson et al., 2006, 2007). Denitrification has been found to decrease with decreasing soil pH, but the ratio of $\text{N}_2\text{O}:\text{N}_2$ produced during the process has been found to increase (Šlmeek and Cooper 2002). Finally, decreased decomposition rates of newly added OM, and increased concentration of C rich ligno-cellulose, have been associated with more acidic soils (van Bergen et al., 1998).

Decreases in soil pH, particularly in the rhizosphere can result from: organic matter mineralisation; CO_2 dissolution; acid rain; fertiliser application and secretion of H^+ ions by plant roots during cation uptake. Under intensive management periodic lime applications prevent soil pH from decreasing. In the absence of liming under permanent extensive grassland management soil pH tends to decrease until equilibrium is reached (Johnston et al., 2007). The pH of the soils from the SRG plots in this study was slightly lower in the 0-10 cm depth range and greater in the 30-40 cm depth range, compared to the paired IM plots, thus the difference in the pH between the upper and lower soil depths was much greater in the SRG plots than the IM plots and there was a statistically significant interaction effect between soil depth and management on soil pH. This significant interaction could be due to the cessation of tillage to the SRGs, preventing the recombining of the soil layers. Processes of acidification, act predominately at the soil surface and in the rhizosphere. When soils are ploughed the more acidic surface soil is combined with the less acidic deeper soil at depth, increasing the pH of the former and decreasing the pH of the latter.

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A lower pH in the surface soil of SRGs could alter nutrient cycling and ES service provision relative to IM plots. Further observations of the rates of nutrient cycling and loss, alongside continued pH measurements at the study sites, would help determine whether surface soil pH will continue to decline over time and what impact this may have on nutrient cycling and ES provision in the long term. The current data show no correlation between the age of the SRG plot and the difference in soil pH from that of the paired IM plot. This observation agrees with the findings of Baer et al. (2002), who found no significant change in soil pH over a chronosequence of restored grasslands ranging from 2-12 years. This lack of an age related effect at the study sites, could suggest that an initial change in soil pH occurs rapidly following conversion to SRG and the cessation of tillage, and then negligible change occurs in subsequent years. If this is the case the impact of soil pH on ES provision from SRGs over time could be small as pH declines are minimal relative to IM fields. Additionally differences between the sites in this study including, soil type, the plant species present and the soil microbial community could be affecting the rate of change in soil pH following conversion from IM to SRG and so masking any age related effects. Longer term sampling from additional sites, preferably where soil pH can be analysed prior to, and for the first few years following conversion, would help determine whether conversion to SRG does have a consistent effect on soil pH, whether soil pH continues to decline at SRGs over time and the extent to which this impacts ES provision.

4.7.4 Assessing the plant species data for each SRG plot

The lowest diversity recorded was in 2010 at plot S3 SRG. The lower diversity could partly be caused by the sowing of a less species rich seed mix in the plot compared to the other SRGs (Section 3.2); the mix used at S3 SRG contained six species of grass but no forb species; using a more species rich seed mix could have helped in establishing higher diversity grassland. The diversity did increase however in 2011; this coincided with an increase in cover from forb species, species richness and mean EI-light, as well as a widening of the EI-light values to include species allocated an EI-light value of 9 (species found only in bright sun). It is likely that the substantial (6%) bare ground at the site in 2010 provided an available niche for light-loving forb species that would other-wise have been shaded out by dominant grass species. One

Chapter 4 Results and discussion from Scottish sites

explanation for the bare ground in 2010 is that it resulted from the drier than average conditions in April- June 2010. A lack of drought tolerant species, and the sandier soil texture compared to the other Scottish sites, may have contributed to the greater % cover of bare ground at site S3 in 2010 compared to the other sites. The impact of drought and the high inter-annual variation in diversity show that, whilst actions such as sowing a richer seed mix may help create a more diverse sward, there are environmental variables that can have a large impact on grassland community development year on year.

The SRG at site S5 differed from the other Scottish sites in that during the years immediately prior to creation of the SRG the plot was under intensive grassland management, as opposed to arable production. As such the established SRG may have been expected to have more in common with the NW SRG plots than the other Scottish plots. There is little evidence in support of this hypothesis however as the diversity, richness and % of seed mix species established in the plot are lower than for the NW plots and much closer to the other Scottish plots. The means and ranges of the EI values are similar to the other Scottish SRGs, suggesting communities of plants occupying similar niches.

The SRG plots at sites S8 and S9 were only one year apart in age, sown with the same seed mix and located within one mile of each other on very similar soil, with a very similar historic use. Despite these similarities, the plant communities at the two plots varied considerably. The % cover from forbs, particularly sown forbs, was much greater in plot S9, whilst the % cover from non-sown grasses was much lower in both years compared to S8 SRG. The diversity, species richness and % of the sown seed mix established were all greater at S9 SRG than S8 SRG. No consistent differences were found in the ranges or means of the EI values of the species at the sites, suggesting the plant species establishing had similar niche preferences. Results from the soil chemistry analysis at the site (Section 4.3), show that at comparable times and depths concentrations of available soil N and P were similar at both the SRGs, suggesting the differences in plant species diversity between the two sites are unlikely to be driven by differences in soil chemistry. The main driver for the differences in diversity, is likely to be the difference in management between the two

plots; S8 SRG was cut twice a year and not grazed (Section 3.2), whilst S9 SRG was cut in March and then grazed at the end of August in both 2010 and 2011. Selective grazing and disturbance by cattle have been found to encourage increased diversity in less intensively managed grasslands (Olf and Ritchie, 1998). Evidence from this study supports these findings and suggests that biodiversity provision may be enhanced by low intensity grazing in preference to cutting alone.

4.7.5 Interpreting mean EI-N in relation to soil available N

The mean EI-N value of plant species at a site has been found to correlate equally to available N and the availability of P and K (Diekmann 2003). All plots in this study contained species ranging from those found on very infertile / infertile soils (EI-N of 2 or 3), to those found on richly / extremely fertile soils (EI-N of 7-9). This wide range indicates a patchy availability of nutrients across the sites, which is in concurrence with the large intra-plot variations found in available N and P at the Scottish sites (Section 4.3)

The mean EI-N values for these plots were similar to those published by other authors; Stevens et al. (2012) studied experimental sites that had been abandoned without fertiliser additions for 15 years and found a mean EI-N of 4.91 at sites with a history of high N addition ($200 \text{ Kg N ha}^{-1} \text{ yr}^{-1}$), compared to a mean EI-N of 4.49 at sites with a history of no or low N additions (less than $25 \text{ Kg N ha}^{-1} \text{ yr}^{-1}$). Critchley et al. (2007) found a mean EI-N of 4.54 to 4.66 in species rich hay meadows in the UK (according to the National Vegetation Classification), compared to a mean EI-N of 4.87-4.89 in degraded hay meadows. The mean EI-N for the plots in this study were mainly within the range of, or greater than the values found by Stevens et al. (2012) in previously fertilised plots and Critchley et al. (2007) in degraded plots. The mean EI-N at plot S8 SRG was 4.63 in 2010, but this increased by 2011 to a much higher value of 5.18, whilst the mean EI-N at plot S9 SRG was an intermediate value of 4.71 in 2010, which decreased to a low value in 2011 of 4.47. Where values fluctuate between years, it could indicate permanent shifts in a community, with species common to low-fertility soils becoming more or less dominant. Alternatively the changes could be a result of temporary shifts in the species present following disturbance, for example the creation of bare ground patches at plot S9 SRG

following drought in 2010, which left available space for colonisation by species which may be naturally less competitive in nutrient rich habitats. Such shifts could be short lived as more competitive species come to dominate again in future years.

4.7.6 Assessing the success of the SRGs in providing enhanced biodiversity

No specific targets for biodiversity provision, against which success can be measured, are set for created SRGs. The SRG plots in this study were successful in providing a much greater diversity than the paired IM plots, which were either crop monocultures or very low diversity grasslands. However, if the sown seed mix is taken as a list of target species then there was partial but not complete success, with the % of seed mix established at sites ranging from 23.1 - 66.7%. If the creation of species rich grasslands is viewed as an attempt to recreate the species rich hay meadows that have been lost from much of Europe (Garcia, 1992), then these created SRG sites must be compared against high diversity sites that remain under traditional, non-intensive management. One study in Sweden looked at traditional hay meadows and recorded SDIs of 2.56-3.71 and mean EI-Ns ranging from 2.3 – 4.5 (Linusson et al., 1998). Janssens et al. (1998) studied old, permanent grasslands and recorded Shannon diversity indexes ranging from 0.5 – 5, with the diversity at the majority of low fertility sites being greater than 2.5. Most of the plots in this study fail to achieve a diversity as high as any of the plots described by Linusson et al. (1998) or most of those studied by Janssens et al. (1998), and have a higher mean EI-N, suggesting the communities contained species favouring more nutrient rich environments than those from these traditional hay meadows.

4.7.7 The effect of the cessation of intensive management on soil N₂O fluxes

The positive correlation between the difference in BD at paired IM and SRG plots and the age of the SRG could indicate an important change in soil physical structure has occurred over time in the SRG plots. The cessation of intensive management practices, including the use of machinery, along with the increased additions of organic matter (Horn et al., 1995) and the development of a permanent grass sward with a root system which can help loosen compacted soil (Gyssels et al., 2005), could all be contributing to this observed decrease in BD over time at the SRG plots. Decreasing BD could decrease the risk of N₂O emissions from the soil over time, as

the increased availability of pore spaces in less dense soil, is likely to reduce the risk of waterlogging and lead to a lower % soil WFPS, so fewer anaerobic sites at which denitrification can occur. There is some evidence to support this hypothesis, as site S3, where the soil BD was greater in the SRG plot than the IM plot, was the only site where the SRG plot was found to have a significantly greater soil WFPS % on any gas sampling occasion. In contrast, at the other three sites, where the soil BD in the SRG plots was lower than in the IM plots, the % WFPS was significantly greater in the IM plots compared to the SRG plots on some sampling occasions.

Other authors have found N_2O emissions from agricultural soils to be limited when soil WFPS is <60% (Dobbie et al. 1999; Bateman and Baggs 2005; Russer et al. 2006). There were several occasions at all sites where the WFPS at the time of gas sampling was below 60%. Even when the WFPS was greater than 60% the N_2O emissions from all plots in this study were generally very low or negligible. The only date on which the N_2O flux from any of the plots was greater than $20 \text{ g N ha}^{-1} \text{ day}^{-1}$ was on 11 May 2011, when the greatest fluxes observed throughout the study were measured from IM plots at sites S8 and S9. Both IM plots received 230 kg ha^{-1} of ammonium nitrate fertiliser on 19 April. It is likely that the rainfall event that occurred on 8 May 2011, which was recorded as 16 mm of rainfall at the Greenlaw met. station (Figure 4.31), combined with the addition of N fertiliser at the end of April, created favourable conditions for N_2O fluxes (Clayton et al., 1997).

The very low, and occasionally negative, N_2O fluxes recorded from the Scottish sites are similar to the fluxes found at the NW sites (Section 5.5). Other authors have also found N_2O emissions peaks to be infrequent and short lived from agricultural sites (Neftel et al., 2007), with negative fluxes commonly measured (Chapuis-Lardy, et al., 2007). The monthly rainfall totals and MDmin / MDmax in the Scottish Borders in 2010 and 2011, were generally closer to the 30-year means than at the NW sites (Section 5.1), so the N_2O fluxes measured are likely to be closer to those expected in a typical year.

The rate of emission of the greenhouse gas N_2O seems to be similar for SRGs and IM plots. However the infrequent flux measurements mean that definite conclusions about the potential difference in net yearly N_2O fluxes from the IM and SRG plots

cannot be drawn. Future sampling should involve N₂O flux measurements being made at least once a week from each site, preferably more regularly following fertiliser additions. The large intra-plot variation in soil WFPS % and available N concentration, as well as in the N₂O flux measurements themselves, highlight the large potential and realized spatial variability in N₂O fluxes and suggest the need for a greater number of chambers in each plot. Increasing the number of chambers to five in each 11 m x 11 m plot, to equal the number of soil sampling points, would help improve the accuracy of any calculated yearly N₂O flux total for comparison between IM and SRG plots.

4.8 Conclusions

The data collected show that soil chemical properties are very similar between the SRG and IM plots, suggesting conversion to SRG results in little change to soil chemistry for at least 10 years following conversion. The data also support the hypothesis that significantly enhanced biodiversity provision from SRG sites up to 10 years is unlikely and suggest this is, in part, due to high residual soil fertility. Whilst inferences, based on soil chemistry, can be made about the potential effect of conversion to SRG on nutrient cycling and associated ES provision, the data on nutrient fluxes themselves are limited. Attempts to measure nutrient concentrations in soil leachate were unsuccessful. Suggestions for an alternative method to collect soil leachate are provided in Chapter 6. Measurements of N₂O fluxes were made, and on most occasions showed equally low fluxes from both IM and SRG sites. However the observation on one occasion of substantial N₂O emissions from two of the IM plots shows the potential for occasional large fluxes, most likely associated with fertiliser application, which could result in significantly greater yearly N₂O emissions from IM plots compared to the SRG plots. Overall, the low frequency of N₂O flux sampling, and the limited number of sampling chambers installed in each plot, have prevented a detailed conclusion as to how conversion to SRG affects the regulation of soil N₂O emissions for the first 10 years following conversion. To address this limitation of the data from the Scottish sites, further N₂O flux sampling was carried out at the IM and SRG plots in NW Devon. At NW, the greater accessibility of the sites and ability to obtain full and accurate details on management activities, meant it

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was possible to obtain a higher quality dataset, from which more robust conclusions could be drawn. The data from NW are presented in the following chapter.

Chapter 5 Results and discussion of data from North Wyke

This chapter summarizes all the data obtained from the field Sites at NW Devon. A site description and methods are given in Chapter 3, results are compared between SRG plots and paired IM plots, and used to assess the potential for enhanced ES provision from the SRG.

The first data presented are climate data, including daily rainfall total, and minimum and maximum temperature for the site. Climate can have an impact on soil physical properties such as temperature and water content, which can in turn affect plant and microbial communities and rates of nutrient loss through gaseous emissions and leachate. The climate conditions for the three years of the study period are compared to the 30-year mean for the site.

The next data presented are from plant species analysis, carried out in summer 2010 and 2011. A key target of created SRGs is that they enhance plant biodiversity, the plant community established at a site can be an indicator of fertility, which itself can influence provision of other important ESs.

The plant community has a strong influence on the soil microbial community and vice versa (Section 2.3), as such data from PLFA analysis of the soil microbes at the IM and SRG plots will follow the plant species data.

The microbial community is responsible for key nutrient cycling processes, including those which regulate the rate of mineralisation, leaching and release of the potent greenhouse gas N₂O (Section 2.3). Soil WFPS, temperature and available N concentration can also strongly influence N₂O fluxes (Chapter 2), results from measurements of these key soil properties are presented, followed by the results from N₂O flux measurements. The measured N₂O fluxes are then compared with results simulated by the UK-DNDC model, to determine the suitability of the model for predicting N₂O emissions under the two management types.

The main source of nutrient loss from soils is in leachate and run-off (Aarts et al., 2000). Limited success was achieved when attempting to collect soil water samples

to measure dissolved nutrient concentration in soil water, but the results from the samples collected will be briefly presented.

5.1 Climate data

To put the N₂O flux data into context this section presents rainfall and air temperature data from the automated weather station (AWS) located in the Burrows field (NGR SX 659983 - No. 8836) at Rothamsted Research NW. The station uses a Munro R100 tipping bucket rain gauge (Munro Meteorological, Woodford Green, UK), and an MK 4a platinum electrical resistance thermometer (MK Electric, Basildon, UK). Also presented are measurements of soil surface temperature (0-2 cm depth) taken at the study plots for 2010-2012 and soil WFPS for 2012.

5.1.1 Analysis of climate data

Monthly mean rainfall and temperature data were analysed to compare the climate during the study period (2010-2012) with the mean climate data for the last 30-years (1982-2011). The 30-year mean monthly rainfall totals and MDMin / MDMax temperature, along with the corresponding standard deviations (SDs) were computed from raw daily rainfall and temperature data using logic function based codes in Microsoft excel. Any monthly mean rainfall total or MDMin / MDMax for 2010-2012 that differed from the 30-year mean by ≥ 2 times the SD was considered significantly different; any measurement differing from the 30-year mean by ≥ 1 SD is also mentioned as being slightly significant. Daily temperature data for the study period are also presented alongside measured soil temperatures, to illustrate how soil temperature varied with air temperature. Daily rainfall data for 2012 are presented alongside soil WFPS data collected in that year, to show how WFPS responds to rainfall events. Data for 2012 only continue until June, when data collection ended.

5.1.2 Comparing monthly rainfall and temperature data for 2010-2012 with 30-year means.

During the study period, rainfall was substantially below the 30-year mean, apart from two very wet months in April and June 2012. In 2010 and 2011 every month received less rainfall than average for the 30-year period (Figure 5.1). In February, June, August and November 2010 the rainfall was less than 1 SD from the 30-year mean, in the other eight months of 2010 the difference was greater than 1 SD from

Chapter 5 Results and discussion from North Wyke

the 30-year mean. As a percentage of the 30-year mean December received the least rainfall in 2010, with 2 mm of rain, which was 2% of the 30-year mean, a difference of 1.99 times the SD. November and June were the only months to receive more than 50% of their 30-year mean rainfall total, receiving 64.9 and 54.5% respectively. The total rainfall for 2010 was 389 mm, which was 37.3% of the 30-year mean of 1041.7 mm.

In 2011 the total rainfall in June, July and September was within 1 SD of the 30-year mean, in the other nine months the rainfall total was more than 1 SD lower than the 30-year mean. April received the lowest percentage of its mean monthly total, with 5.5%, which was 1.76 times the standard deviation below the 30-year mean. June and September were the only months to receive > 50% of their 30-year mean monthly rainfall totals; 52.0 and 70.2% respectively. The total rainfall in 2011 was 379.4 mm, 36.4% of the 30-year mean.

In contrast, April 2012 received 258.6% of the 30-year mean rainfall for the month, with a total of 169 mm, and June received 157.2 mm of rain, which was 276.3% of the 30-year mean. The first three months of 2012 were drier than average, with January, February and March receiving 77.1, 28 and 32.5% respectively of their 30-year mean rainfall totals.

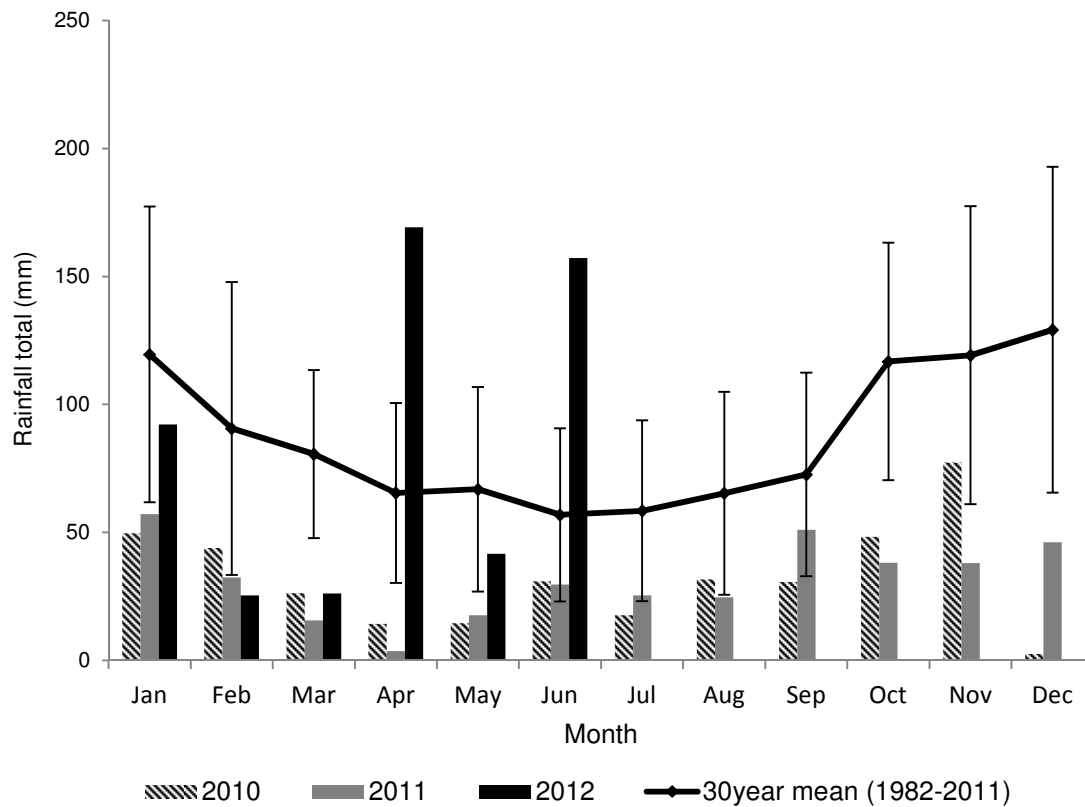


Figure 5.1 Monthly rainfall totals for 2010-2012 alongside 30-year (1982-2011) mean monthly rainfall totals recorded at the NW AWS, error bars show ± 1 SD. 2012 data only included up until June, when data collection for the study ended.

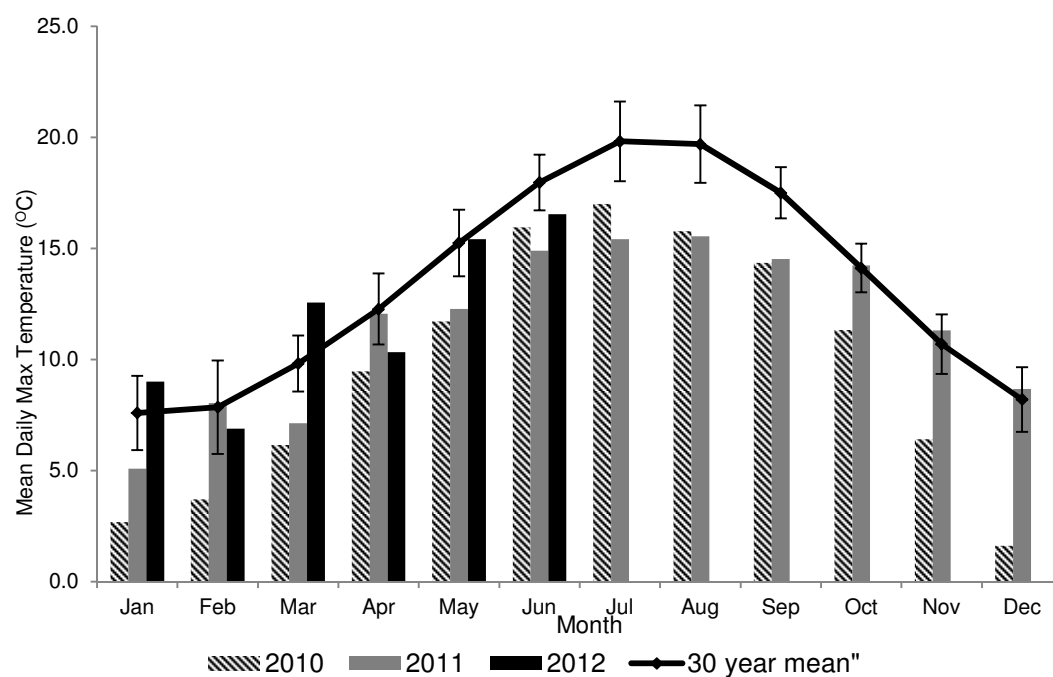
In general maximum daily air temperatures during the study period were slightly lower than the 30-year means, especially in 2010. Overall the range in air temperature appeared to be slightly less than the long term average. In 2010 the MDMax in February, April, June, July and August was more than 1 SD below the 30-year mean, in the other seven months of 2010 the MDMax was more than 2 times the SD below the 30-year mean (Figure 5.2a). Across all 12 months the mean difference between the 2010 and the 30-year mean MDMax was $3.7 (\pm 1.2 ^\circ\text{C})$. The smallest difference was in June, when the 2010 MDMax was $2.0 ^\circ\text{C}$ lower than the 30-year mean and the greatest difference in 2010 was in December, when the MDMax was $6.6 ^\circ\text{C}$ lower than the 30-year mean. The difference between the MDMin in 2010 and the 30-year mean MDMin was also greatest in December (Figure 5.2b), when the MDMin was more than 2 times the SD lower than the 30-

year mean. The MDMin recorded in January, November and December 2010 were also more than 1 SD lower than the 30-year means. In July the MDMin was more than 1 SD greater than the 30-year mean. The MDMin in all other months in 2010 was within 1 SD of the 30-year mean.

In 2011 the MDMax in January, March, May, June, July August and September was more than 1 SD lower than the 30-year mean. The MDMax in the remaining five months was within 1 SD of the 30-year mean. In February, April, September, October, November and December 2011 the MDMin was more than 1 SD greater than the 30-year mean. The MDMin in July was more than 1 SD lower than the 30-year mean. In all other months in 2011, the MDMin was within 1 SD of the 30-year mean.

2012 was generally warmer than 2010 and 2011; in March the MDMax was more than 2 times the SD greater than the 30-year mean. The MDMax in April and June was more than 1 SD less than the 30-year mean.

a)



b)

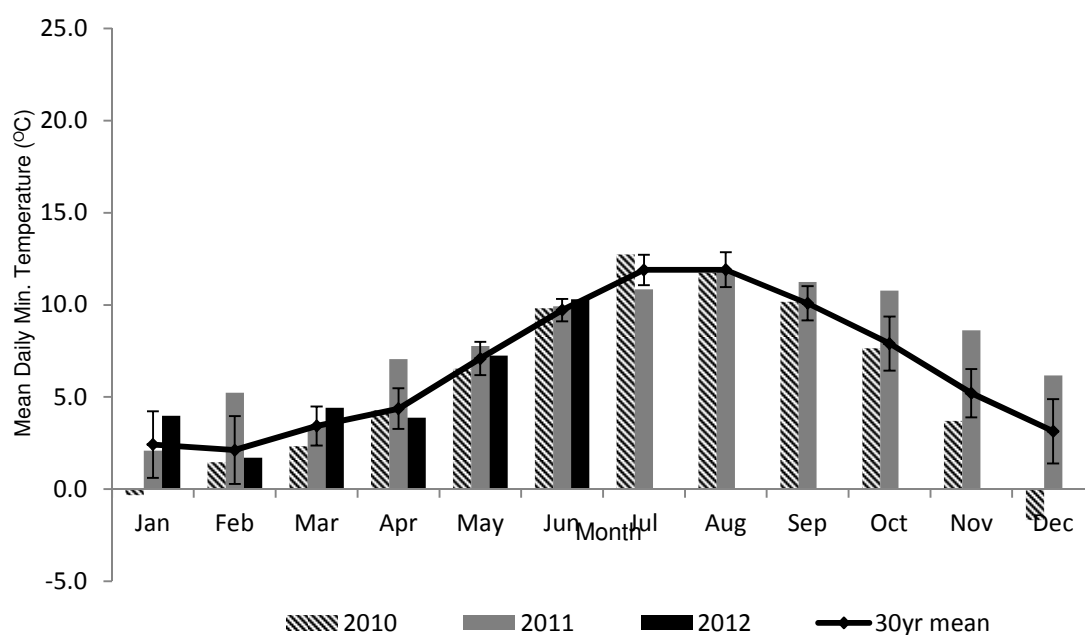


Figure 5.2 a) Mean maximum daily temperature and b) Mean minimum daily temperature, for each month in 2010-2012 alongside 30-year mean, error bars $\pm 1SD$.

5.2 Plant species survey

This section presents the percentage cover from different plant groups for each SRG plot in 2010 and 2011. Plants are grouped according to whether they were present in the seed mix sown on establishment of the SRG (sown) or not (non-sown), and subsequently into grasses, L forbs and NL forbs. Also presented are the species diversity and richness and the % of seed mix species that successfully established for each SRG plot in 2010 and 2011. Finally, the mean and range of EI values for nitrogen (EI-N), moisture (EI-moisture), light (EI-light) and pH (EI-pH) are presented for the species identified in each plot.

5.2.1 Analysis of plant species data

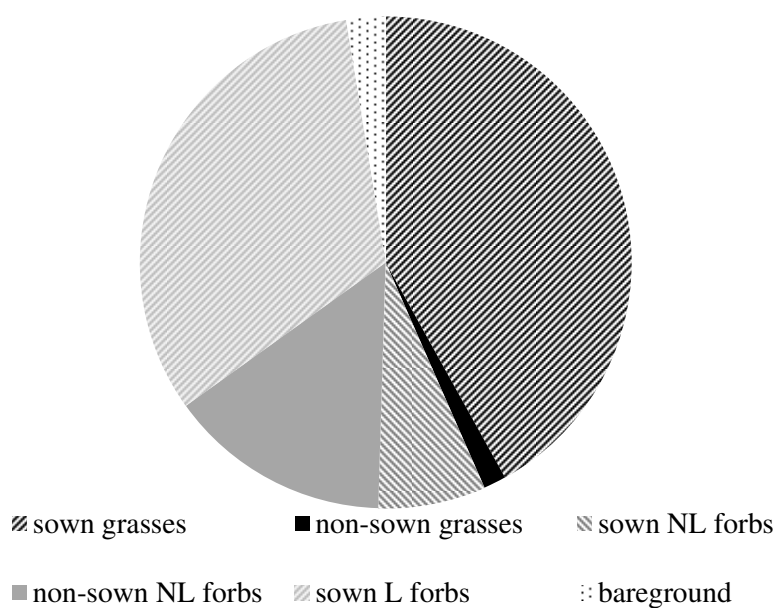
Plant species data for the NW SRG plots were analysed in the same way as the data from the Scottish SRG plots (Section 4.4.1) in order to identify the success of sown species and how diversity changed over the two years of sampling (2010 and 2011). Mean ($n=5$) % cover for each plot was calculated, as was the range and mean of the EIs for the established species in each plot. The Shannon diversity index (H) was calculated for each plot, using the mean % cover to determine the P_i for each species (Equation 11).

5.2.2 Percentage cover

Across all the NW SRG plots in 2010 and 2011, the group of plants providing the greatest proportion of cover was the sown grasses and at all plots the % cover provided by the sown grasses increased from 2010 to 2011. Very little cover, less than 4%, was provided by non-sown grasses in any plot in either year. All legume species established had been present in the seed mix. As a % of the total cover provided by all forbs, the L-forbs made up between 18 and 28%, with the exception of plot 1 in 2010 where they made up 60%.

In 2010 the percentage cover provided by sown and non-sown forbs was fairly similar across all the plots. In 2011 the percentage cover provided by the non-sown forbs decreased to less than 6% of the 2010 cover in plots SRG 2 – 4 and less than 13% in SRG 1, whereas the % cover provided by the sown grasses changed little in plots SRG 1, 3 and 4 and decreased by 50% in SRG 2 between 2010 and 2011.

a)



b)

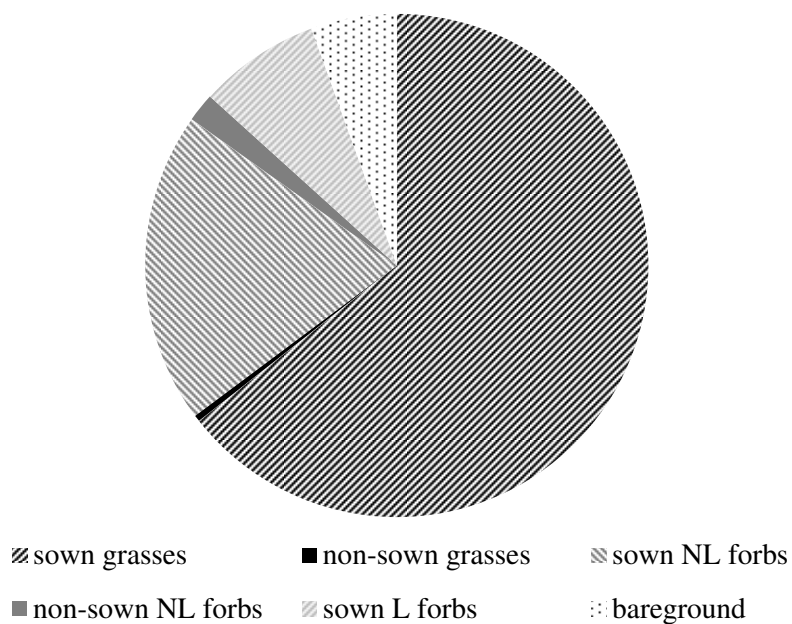
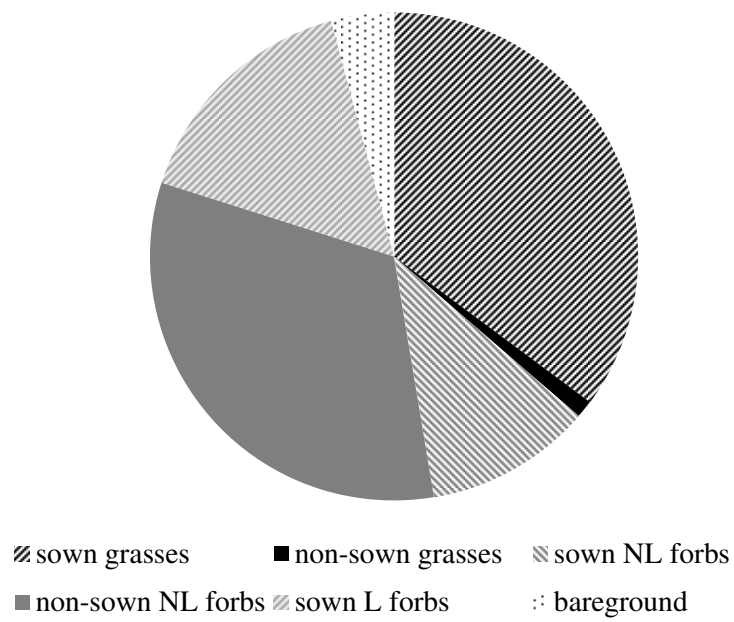


Figure 5.3 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown), leguminous (L) and non-leguminous (NL) forbs that were either sown or non-sown and bare ground at NW SRG plot 1 a) in 2010 and b) in 2011.

a)



b)

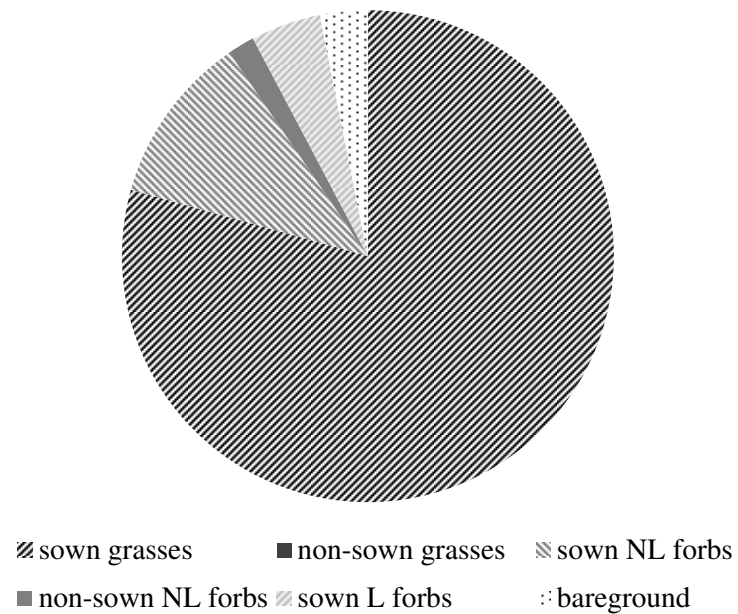
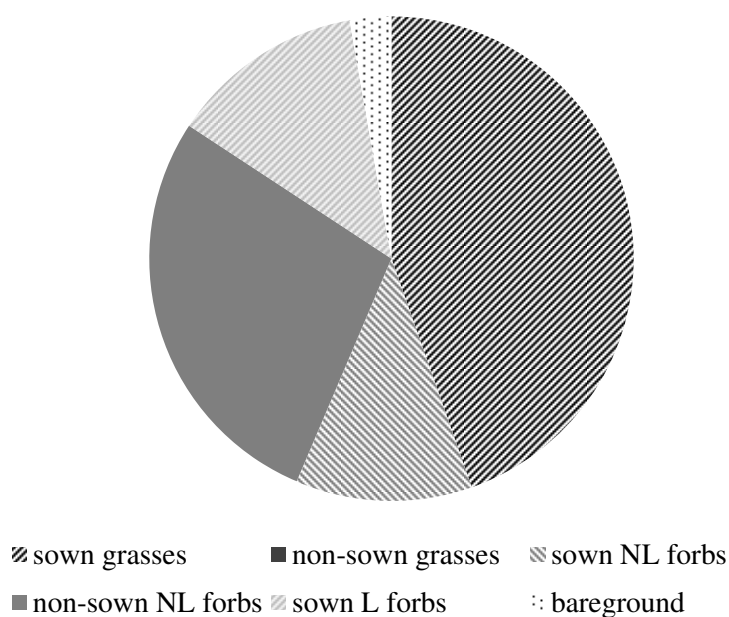


Figure 5.4 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown), forbs that were either sown or non-sown and bare ground at NW SRG plot 2 a) in 2010 and b) in 2011.

a)



b)

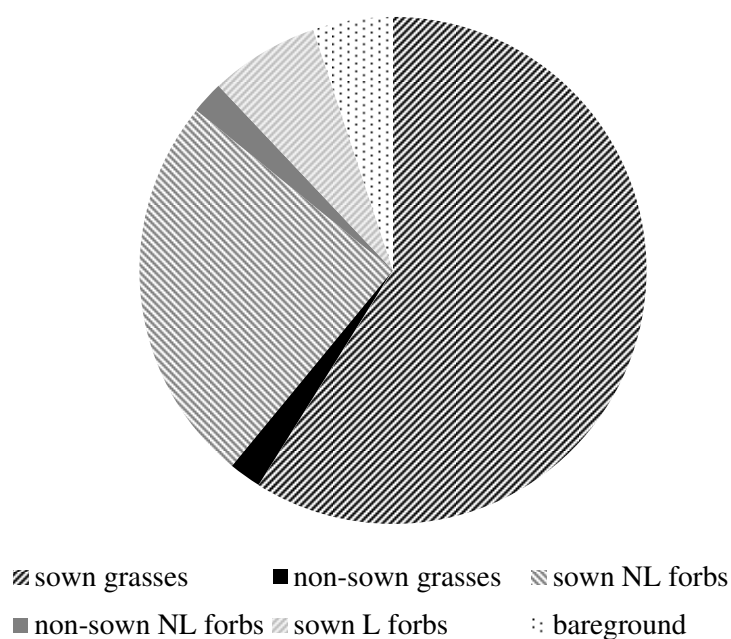
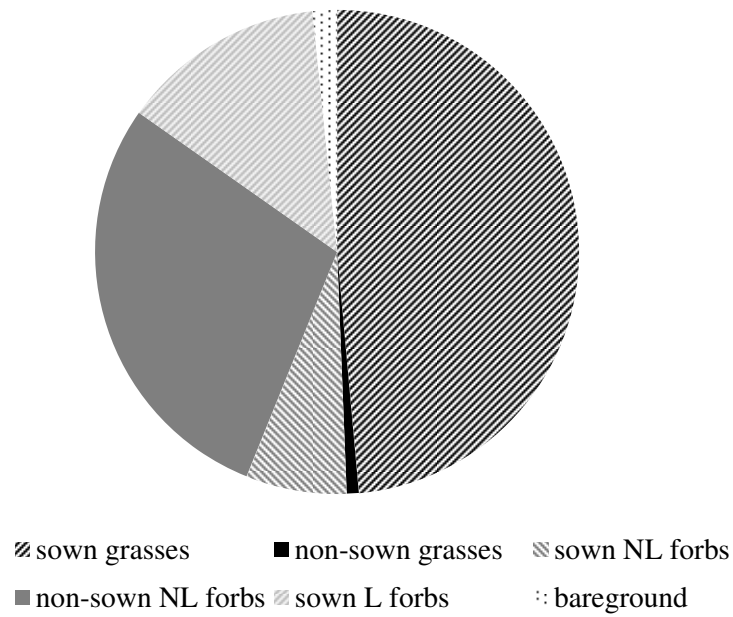


Figure 5.5 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown), leguminous (L) and non-leguminous (NL) forbs that were either sown or non-sown and bare ground at NW SRG plot 3 a) in 2010 and b) in 2011.

a)



b)

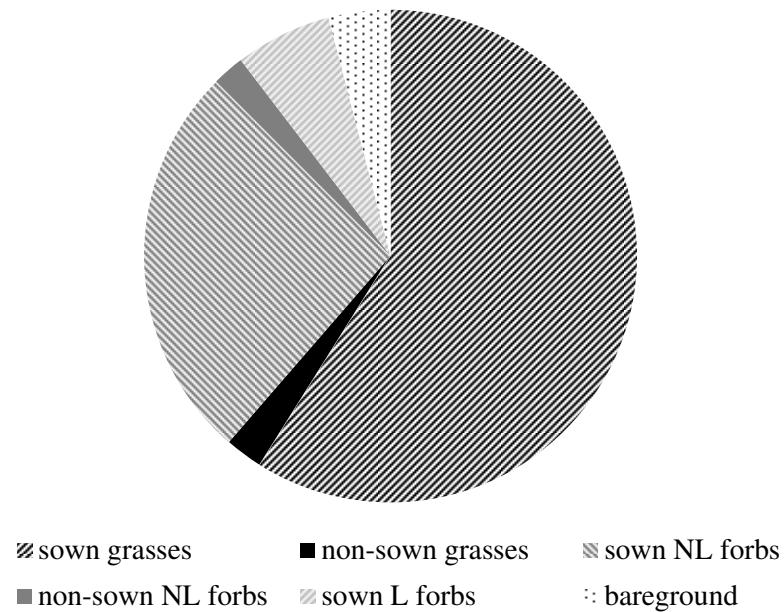


Figure 5.6 Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown), leguminous (L) and non-leguminous (NL) forbs that were either sown or non-sown and bare ground at NW SRG plot 4 a) in 2010 and b) in 2011.

5.2.3 Species diversity and richness

In general the species diversity, richness and % of seed mix established in a given year was very similar in all four replicate plots (Table 5.1). In 2010 the SDI ranged from 2.04 in plot 1 and 2.14 in plot 3, in 2011 the SDI in all plots was less than in 2010, the lowest SDI was in SRG 2 (1.54) and the highest SDI was in SRG 4 (1.97). The mean (n=4) decrease in the SDI across the NW SRG plots between 2010 and 2011 was 0.308 ± 0.172 .

Between 11 and 13 (61.1 and 76.5%) of the 18 sown species were identified in the four plots in 2010 and between 11 and 12 (61.1 and 66.7%) in 2011. In 2010 the mean percentage of seed mix species established was $66.8 \pm 6.4\%$ and in 2011 it was $65.3 \pm 2.8\%$. The only plot to show a change in the % of seed mix species established between 2010 and 2011 was SRG 4, which showed a decrease from 76.5 to 66.7%, equivalent to 1 fewer seed mix species being identified.

The mean (n=4) species richness for the plots was 17.5 ± 1.91 in 2010 and 17.25 ± 1.5 in 2011. Species richness decreased in SRG plots 1, 2 and 4, between 2010 and 2011, whilst it increased in SRG 3.

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Table 5.1 Summarizing the diversity, species richness and the percentage of all species sown in the seed mix that were established in each NW SRG plot in 2010 and 2011. Results are from identification of all vascular plant species within five 1 m x 1 m quadrats in each plot, quadrats were located using random numbers.

Plot	Shannon diversity index		% of seed mix species which have established		Total species richness	
	2010	2011	2010	2011	2010	2011
NW SRG						
plot 1	2.04	1.73	61.1	61.1	17	16
NW SRG						
plot 2	2.06	1.54	66.7	66.7	19	16
NW SRG						
plot 3	2.14	1.84	66.7	66.7	15	19
NW SRG						
plot 4	2.07	1.97	76.5	66.7	19	18

5.2.4 Ellenberg indicator values of established plant species

The widest range in EI-N was observed in SRG 2 in 2011, which contained species with EI-N ranging from 2-9. The modal range of EI-Ns for all four plots was 2-7 (Table 5.2). In 2010 the EI-N range in all the plots was 2-7. The range in EI-N values for N narrowed by 2 in SRG 1 and SRG 4, and widened by 2 in SRG 2, between 2010 and 2011. The mean EI-N was ≥ 5 in both years for all the plots except SRG 4 in 2011. SRG 4 was the only plot to show a decrease in mean EI-N between 2010 and 2011, the mean EI-N of 5.22 in SRG 4 was the greatest of the four plots in 2010, whereas in 2011 the lowest mean EI-N of 4.78 was recorded at SRG 4.

The modal range in EI-light was 6-8; this was the range at all the SRG plots in 2010 and all but one in 2011; no deviation from this modal range greater than 1 EI unit was recorded. The mean EI-light for all the NW plots was very similar in 2010 varying by < 0.05 EI units. All the plots showed an increase in mean EI-light from 2010 to 2011, plots SRG 1-3 showed a small increase in mean EI-light (between 0.08 and 0.04 EI units) and the three mean values remained within 0.02 EI units of one another. Plot SRG 4 showed a greater increase in mean EI-light of 0.28 EI units between 2010 and 2011.

The values for EI-moisture for all four plots ranged between 4 and 7 in both 2010 and 2011. The mean EI-moisture did vary between plots; in 2010 the greatest mean EI-moisture of 5.17 was recorded at SRG 4, the lowest mean EI-moisture of 4.93 was recorded at SRG 3. In 2011 the greatest mean EI-moisture was recorded in SRG 1 (5.13) and the lowest in SRG 2 (4.88).

The modal range in EI-pH for the four plots was 5-8; the modal EI-pH range did not differ by more than 1 EI unit for any plot in either year. SRG 4 was the only plot to show a change in EI-pH range between 2010 and 2011, from 4-8 to 6-8. In the other three plots EI-pH range was the same in both years. SRG 4 also showed the greatest change in mean EI-pH between 2010 and 2011, with an increase of 0.18 units. The other three plots all showed smaller increases in mean EI-pH between 2010 and 2011.

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Table 5.2 Mean and range of Ellenberg indicator (EI) values for nitrogen (N), light, moisture and pH of all plants identified within the five 1m x 1 m quadrats in each SRG plot in 2010 and 2011. Quadrats located using random numbers.

Plot	N - Range of EI values		N - Mean of EI values		Light - Range of EI values		Light - Mean of EI values		Moisture - Range of EI values		Moisture - Mean of EI values		pH - Range of EI values		pH - Mean of EI values	
	201	201														
	0	1	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
NW SRG																
1	2-7	4-7	5.06	5.5	6-8	6-8	7.12	7.19	4-7	4-7	5.12	5.13	5-7	5-7	6.35	6.38
NW SRG																
2	2-7	2-9	5.05	5.25	6-8	6-8	7.11	7.19	4-7	4-7	5	4.88	5-8	5-8	6.42	6.5
NW SRG																
3	2-7	2-7	5	5.06	6-8	6-8	7.13	7.17	4-7	4-7	4.93	5.06	5-8	5-8	6.4	6.44
NW SRG																
4	2-7	3-6	5.22	4.78	6-8	7-8	7.16	7.44	4-7	4-7	5.17	4.89	4-8	6-8	6.26	6.44

5.3 PLFA analysis of soil microbial community

This section provides detail of how data generated from PLFA analyses were used to determine changes in soil microbial community composition. The biomarkers used for the major microbial groups (see Table 5.3) are identified and calculations used to determine their concentrations are given, followed by details of the statistical treatment of these concentration data. The results are reported as total PLFA concentration, fungal:bacterial ratios and Gram positive bacteria (G+ve):Gram negative bacteria (G-ve) biomarker concentration ratios and actinobacteria biomarker concentrations compared between IM and SRG plots. Comparisons are also made between sampling dates to identify any significant temporal changes in biomarker concentrations.

5.3.1 Analysis of PLFA data

The sum of the concentrations of all biomarkers for a specific taxonomic group was calculated and used to compare the relative size of the population of that group in different soil samples. Table 5.3 gives the PLFAs which were identified and used as biomarkers for the following major microbial groups; fungi, G+ve bacteria, G-ve bacteria and actinobacteria (a sub-group of G+ve bacteria). The concentrations of non-specific PLFAs were also determined to provide an estimate of the size of the total microbial population. In terms of the notation, the length of the C chain is given followed by a colon then the number of double bonds and the position (number of C atoms) of the double bond from the methyl end (ω) of the molecule, the letters *c* and *t* indicate the *cis* / *trans* isomerism respectively of the double bond. The prefixes *i* and *a* identify *iso* and *anteiso* branched C chains, 10-Me is used where a methyl group is located on the 10th C atom from the carboxyl end of a molecule and *cy* indicates a cyclopropane fatty acid.

Table 5.3 The PLFA biomarkers used to estimate relative population sizes of key microbial groups, adapted from Dungait et al. (2011).

Microbial group	Biomarkers
Fungi	18:2 ω 6,9
G+ve bacteria	<i>i</i> 14:0, <i>i</i> 15:0, <i>a</i> 15:0, <i>i</i> 16:0, <i>i</i> 17:0, <i>a</i> 17:0, <i>i</i> 20:0, <i>a</i> 20:0
G-ve bacteria	16:1 ω 9, 16:1 ω 7c, 16:1 ω 7t, <i>cy</i> 17:0, 18:1 ω 9, 18:1 ω 7, <i>cy</i> 19:0
Actinobacteria	10-Me18:0
Non-specific	14:0, 15:0, 16:0, 18:0

Once the peaks from the GC/MS had been identified the biomarker peak areas were compared to that of the internal C19 *n*-alkane standard and used to calculate the concentration of each of the biomarkers in the soil sample, using equation 12.

$$\frac{\text{Biomarker peak area}}{\text{C19 standard peak area}} \times \text{standard volume } (\mu\text{l}) \times \text{standard conc. } (\mu\text{g } \mu\text{l}^{-1}) \div \text{mass of dry soil sample } (\text{g}) = \text{biomarker conc. } (\mu\text{g } \text{g}^{-1}) \quad (12)$$

In total PLFA analysis was carried out on six soil samples, one from each of the SRG and IM plots, for each of four dates (see appendix E for table of individual PLFA concentrations for each soil sample). All the reagent blanks were found to be clear of FAMES, with the only detectable peak being that of the C19 *n*-alkane standard, this control verified that the samples were not contaminated.

For each soil sample, the total PLFA concentration was calculated and then the ratios of fungal:bacterial biomarkers and G+ve:G-ve biomarkers and the concentration of actinobacteria biomarkers. T-tests were carried out to identify whether any significant differences were observed in any of these calculated parameters between the three SRG plots and the three IM plots on any individual sampling date

5.3.2 Total PLFA concentration

Total PLFA concentrations in the IM and SRG soils were compared. Across the four sampling occasions the mean (n=3) PLFA concentrations of the soil samples ranged from 5.06 – 6.21 $\mu\text{g } \text{g}^{-1}$ dry soil and 8.97–10.6 $\mu\text{g } \text{g}^{-1}$ dry soil for the SRG and IM plots respectively. On every sampling date the mean total PLFA concentration of the SRG plots was lower than that of the IM plots (Figure 5.7), this difference was significant

on three of the four sampling dates; 5 March ($T(4)=6.32$, $P=0.003$), 11 March ($T(2)=5.35$, $P=0.033$) and 19 April ($T(4)=5.36$, $P=0.006$).

The difference in mean soil PLFA concentration between the two management types was relatively consistent through time. As a percentage of the mean PLFA concentration in IM plot soils, the mean PLFA concentration in the SRG plot soils ranged from 53.8 - 62.3% on 11 March and 19 April respectively. With the exception of the IM plots on 17 May, the PLFA concentrations in the soil were similar for the three replicate plots under each management, resulting in a low SD.

We compared the mean total PLFA concentrations on each of the sampling dates for both the IM and SRG plots to identify any significant temporal changes in the total soil microbial biomass. The mean PLFA concentration in the IM soil samples collected on 11 March ($10.6 \mu\text{g g}^{-1}$ dry soil) was significantly greater than in the samples collected on 5 March ($9.8 \mu\text{g g}^{-1}$ dry soil, $T(4)=3.6$, $P=0.023$). There were no other significant differences between the mean soil PLFA concentrations on different dates for either the IM or SRG plots.

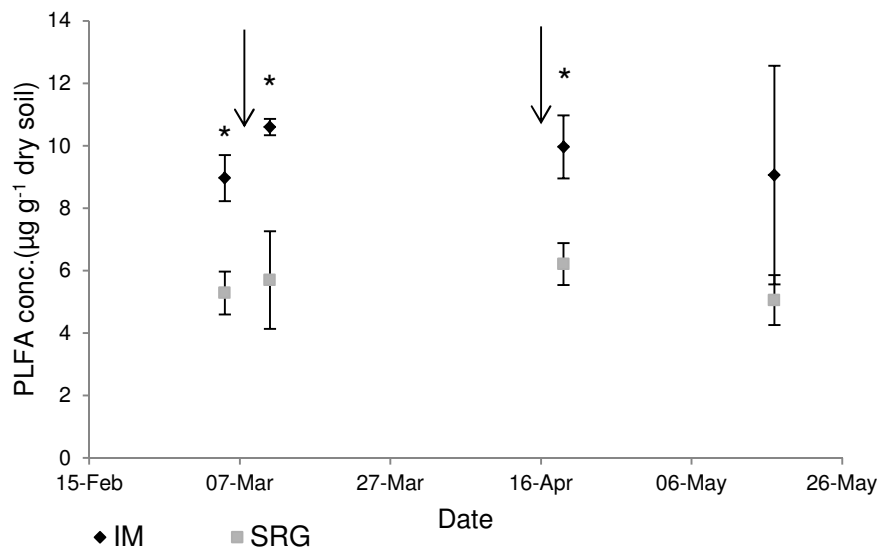


Figure 5.7 Mean ($n=3$) total PLFA concentration in soils collected from IM and SRG plots on four dates in 2012. Error bars show ± 1 SD. An asterisk indicates that the values for the IM and SRG plots differ significantly and arrows show the timing of fertiliser applications to the IM plots on 9 March and 17 April when 400 and 116 kg N ha^{-1} were applied respectively.

5.3.3 Fungal:bacterial biomarker ratio

We compared the mean ($n=3$) fungal:bacterial biomarker ratio in the IM and SRG plots for each sampling date to identify any significant effect of management on the broad taxonomic distribution of the soil microbes. The mean ($n=3$) ratio of fungal:bacterial biomarkers ranged from 0.05-0.1 and 0.02-0.07 in the SRG and IM plots respectively. The ratio was greater in soil samples from the SRG plots compared to the IM plots on all sampling dates (Figure 5.8). This difference was significant on three of the four dates: 5 March ($T(4)=-4.08$, $P=0.015$), 11 March ($T(4)=-3.76$, $P=0.02$) and 19 April ($T(4)=-5.31$, $P=0.006$).

As a percentage of the mean fungal:bacterial biomarker ratio in SRG soils, the mean fungal:bacterial biomarker ratio in the soil from the IM plots ranged from 29.7% on 19 April to 91.8% on the 17 May. As with the total PLFA concentration, the greatest standard deviations of the three replicate plots were observed on 17 May.

The differences in mean fungal:bacterial biomarker concentrations between the IM and SRG plots on all sampling dates were mainly due to smaller bacterial biomarker concentrations in the SRG compared to the IM soils. The mean bacterial biomarker concentration was significantly higher in the IM plots on three of the four sampling occasions: 5 March ($T(4)=6.15$, $P=0.004$), 11 March ($T(2)=5.72$, $P=0.029$), 19 April ($T(4)=6.12$, $P=0.004$). There was no difference in the mean fungal biomarker concentration between the IM and SRG plots on any sampling occasion.

We compared the mean fungal:bacterial biomarker ratios on each of the four sampling dates for both the IM and SRG plots to identify any significant shifts in the taxonomic distribution of soil microbes over time. The mean soil fungal:bacterial biomarker ratio was significantly greater in the IM soils collected on 11 March (0.04) than in those collected on 5 March (0.02, $T(4)=-3.44$, $P=0.026$). This increase in the fungal:bacterial biomarker ratio observed between 5 March and 11 March was due to a greater increase in mean fungal biomarker concentration than in bacterial biomarker concentration between the two dates (Figure 5.9).

The mean fungal:bacterial biomarker ratio in the SRG soil samples collected on 19 April (0.1) was significantly greater than the ratio in the soil samples collected on 5

March (0.05, $T(4)=-6.65$, $P=0.003$) and on 11 March (0.06, $T(4)=-5.34$, $P=0.006$).

The increase in mean fungal:bacterial biomarker ratio in the SRG soils between 5 March and 19 April was due to an increase in fungal biomarker concentration as opposed to a decrease in bacterial biomarker concentration (Figure 5.9).

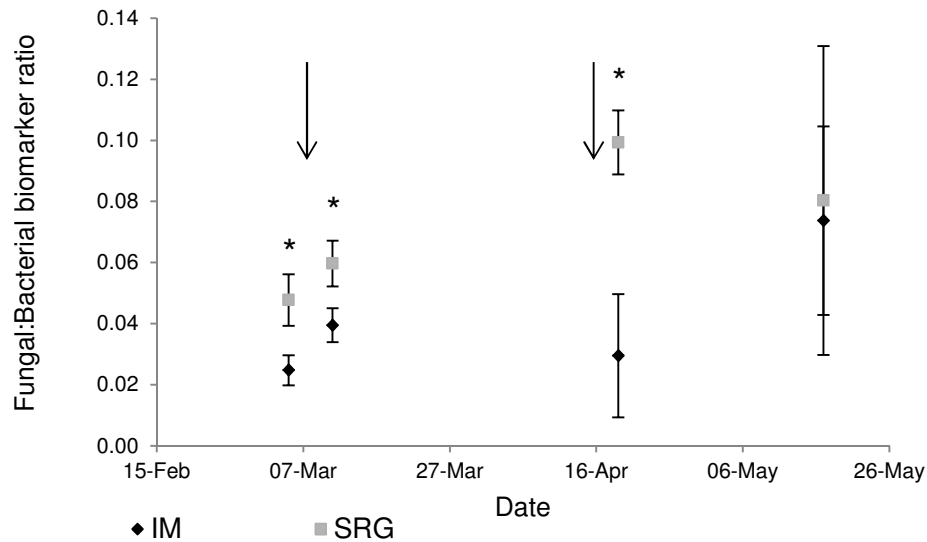
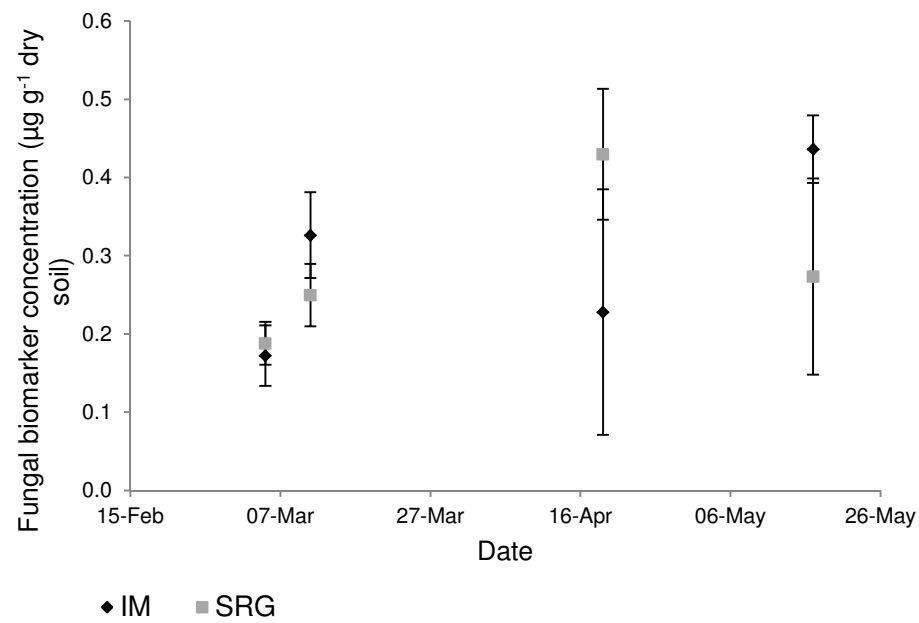


Figure 5.8 Mean ($n=3$) fungal:bacterial biomarker ratio in soils collected from IM and SRG plots on four dates in 2012. Error bars show $\pm 1SD$. An asterisk indicates that the values for the IM and SRG plots differ significantly and arrows show the timing of fertiliser applications to the IM plots on 9 March and 17 April when 80 and 39 kg N ha⁻¹ were applied respectively.

a)



b)

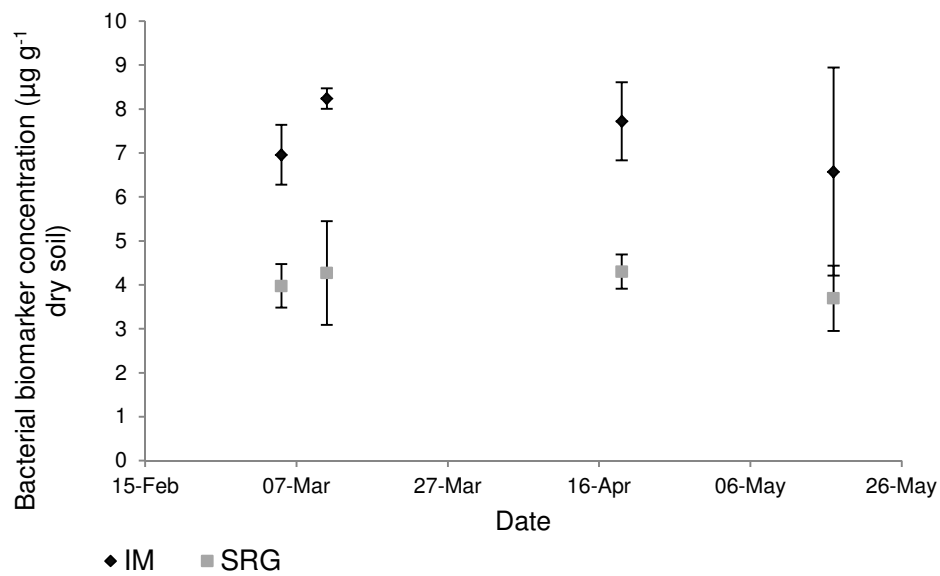


Figure 5.9 Mean ($n=3$) concentration of a) fungal and b) bacterial biomarkers in soil samples collected from IM and SRG plots on four sampling dates in 2012. Error bars show $\pm 1SD$.

5.3.1 G+ve:G-ve biomarker ratio

The ratio of G+ve:G-ve bacterial biomarkers was compared between the IM and SRG plots on each sampling date to identify any significant effect of management on

the structure of the soil bacterial community. The mean (n=3) G+ve:G-ve bacterial biomarker ratios ranged from 0.46 – 0.64 and 0.45-0.58 across the four sampling dates for the SRG and IM plots respectively. Although the the G+ve:G-ve biomarker ratios tended to be slightly higher for the SRG soils compared to the soils from the IM plots, the difference was not significant for any sampling date (Figure 5.10).

The mean G+ve:G-ve biomarker ratios on the four sampling dates was compared for the IM and SRG plots to identify any significant shifts the bacterial community composition over time. The mean G+ve:G-ve biomarker ratio in the SRG soil samples collected on 19 April (0.64) was significantly greater than the ratio in the soil samples collected in the SRG plots on 5 March (0.46, $T(4)=-3.75$, $P=0.02$) and 11 March (0.48, $T(4)=-4$, $P=0.016$). This increase in G+ve:G-ve biomarker concentration between 5 March and 19 April was due to both a slight increase in G+ve biomarker concentration and a slight decrease in G-ve biomarker concentration in the SRG soils (Figure 5.11).

5.3.2 Actinobacteria biomarker concentration

The soil actinobacteria biomarker concentration in the IM and SRG plots was compared for each sampling date, to identify any significant effect of management on the population size of this sub-group of G+ve bacteria. The mean (n=3) actinobacteria biomarker concentration ranged from 0.09 - 0.15 $\mu\text{g g}^{-1}$ dry soil and 0.23 – 0.26 $\mu\text{g g}^{-1}$ dry soil across the four sampling dates for the SRG and IM plots respectively. The mean (n=3) actinobacteria biomarker concentration was greater in the soil samples from the IM plots than the SRG plots (Figure 5.12). The difference was significant on three of the four sampling dates: 5 March ($T(4)=4.36$, $P=0.012$), 11 March ($T(4)=11$, $P=0.0004$), 19 April ($T(4)=5.58$, $P=0.005$), as a % of the mean actinobacteria biomarker concentration in the IM plot soils, the mean actinobacteria biomarker concentration in the SRG soils ranged from 39.9% on 11 March to 60.4% on 19 April.

We compared the mean soil actinobacteria biomarker concentration on each sampling date for the IM and SRG plots to identify any temporal change in the actinobacteria population size. The mean actinobacteria biomarker concentration in the soil samples collected from the SRG plots on 19 April (0.15 $\mu\text{g g}^{-1}$ dry soil) was

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significantly greater than in the soil samples collected on 11 March ($0.09 \mu\text{g g}^{-1}$ dry soil, $T(4)=-2.89$, $P=0.045$).

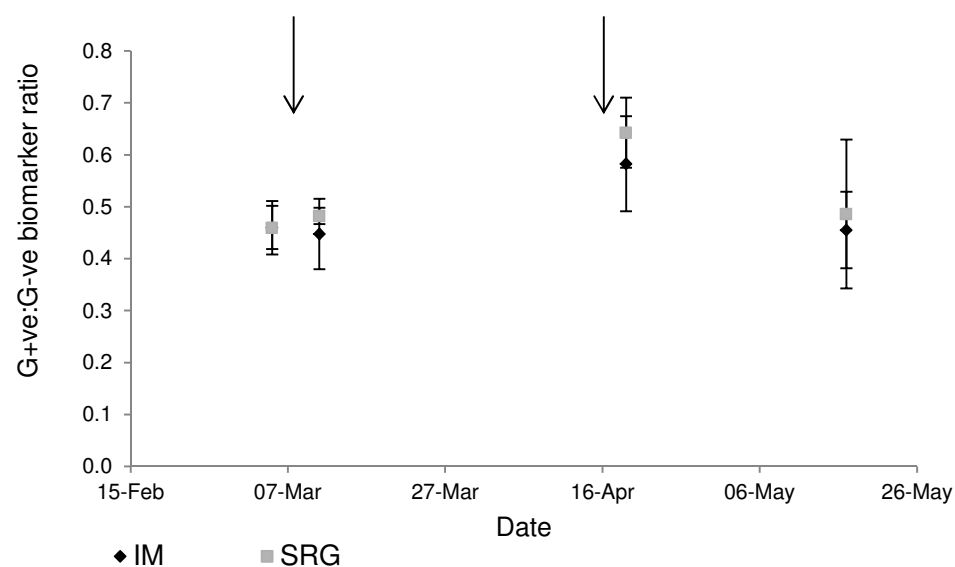
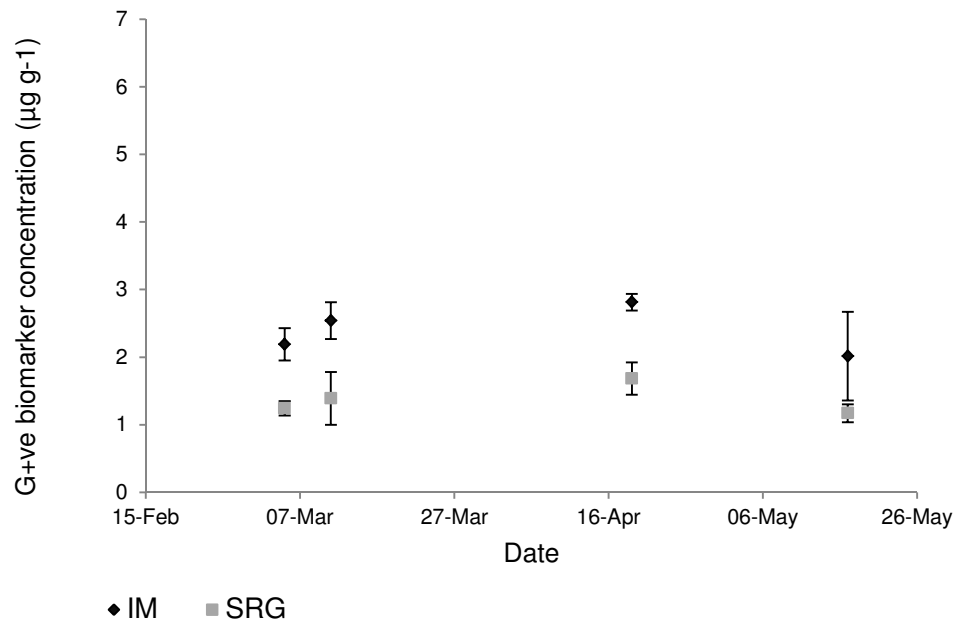


Figure 5.10 Mean ($n=3$) G+ve:G-ve biomarker ratio in soils collected from IM and SRG plots on four dates in 2012. Error bars show $\pm 1SD$. Arrows show the timing of fertiliser applications to the IM plots on 9 March and 17 April when 400 and 116 kg N ha⁻¹ were applied respectively.

a)



b)

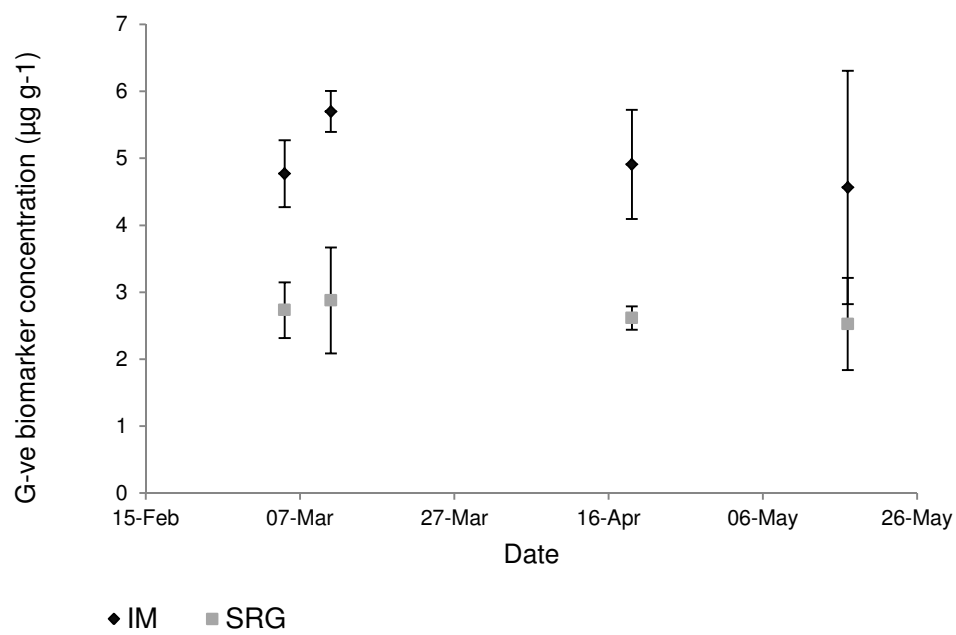


Figure 5.11 Mean (n=3) concentration of a) G+ve and b) G-ve biomarkers in soil samples collected from IM and SRG plots on four sampling dates in 2012. Error bars show ±1SD.

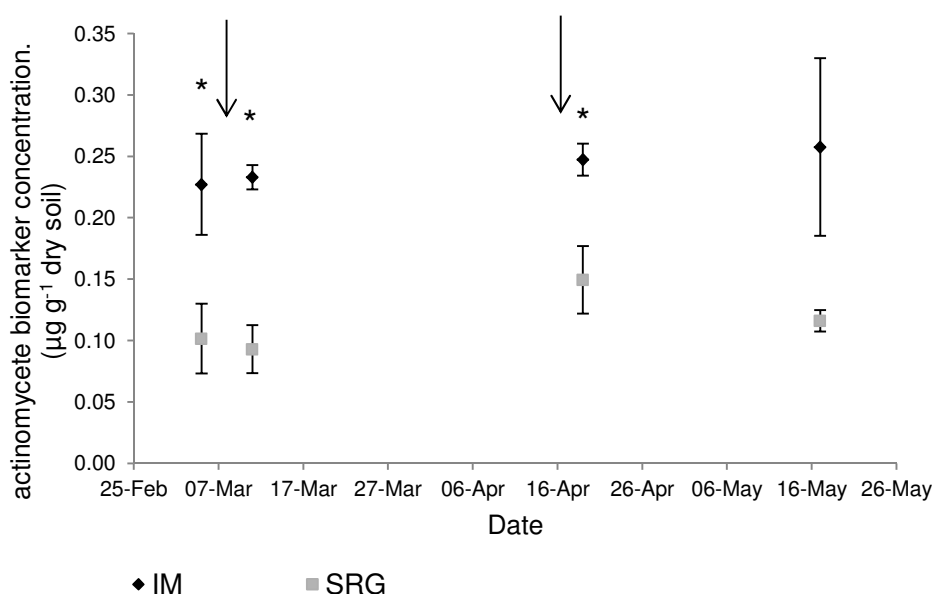


Figure 5.12 Mean ($n=3$) actinobacteria biomarker concentration in soils collected from IM and SRG plots on four dates in 2012. Error bars show ± 1 SD. An asterisk indicates that the values for the IM and SRG plots differ significantly and arrows show the timing of fertiliser applications to the IM plots on 9 March and 17 April when 400 and 116 kg N ha⁻¹ were applied respectively.

5.4 Soil temperature, WFPS and available N

5.4.1 Analysis of soil temperature, WFP and available N data

Soil temperature, WFPS and available NO_3^- -N/ NH_4^+ -N concentration, were analysed separately for each sampling occasion in Microsoft Excel using a two factor ANOVA without replication. The two factors were; management type (IM / SRG) and plot pair (1-4), where paired IM and SRG plots were matched for slope and soil type. Plots were sampled in the same plot number order at both sites. The data were analysed to determine any statistical difference in soil moisture or temperature at the two sites on any individual sampling occasion and to identify whether any of these differences occurred consistently across multiple sampling occasions, which would indicate a possible effect of the management on that soil property.

5.4.2 Soil temperature

Soil temperatures lay within the range of daily air temperatures measured by the Burrows AWS. Since measurements were taken between 10:00 and 14:00, alongside

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gas sampling, the soil temperature was usually closer to the MDMax than the MDMin (Figure 5.13). Soil temperatures were similar at both sites, with no consistent difference between the two management types. There was a significantly greater mean soil temperature in the IM plots on seven occasions and in the SRG plots on eight occasions (two factor ANOVA without replication, $P \leq 0.05$).

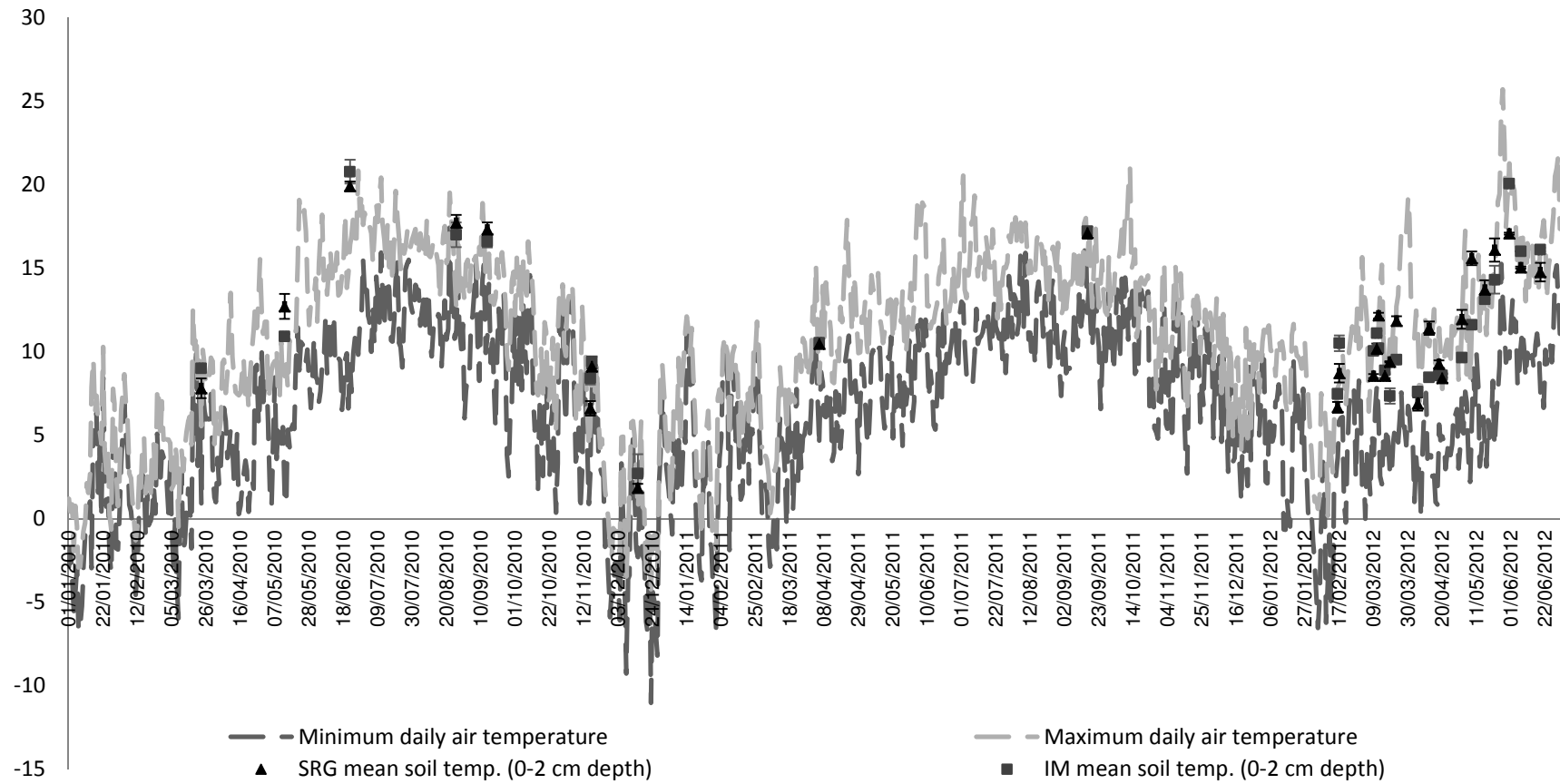


Figure 5.13 Mean (n=4 in 2010/2011) (n=3 in 2012) soil temperature of all SRG and IM plots at time of sampling, error bars ± 1 SD, alongside daily minimum and maximum air temperature.

5.4.1 Soil WFPS (2012)

BD and organic matter content measured on 22 May 2012 (Section 3.7) were used to calculate WFPS. Mean (n=3) BD was $0.95 (\pm 0.03 \text{ g cm}^{-3})$ for the SRG plots and $0.72 (\pm 0.02 \text{ g cm}^{-3})$ for the IM plots. Mean (n=3) organic matter content was 3.83 ($\pm 0.14\%$ mass) for SRG plots and 5.26 ($\pm 1.20\%$ mass) for IM plots. Mean (n=3) soil porosity values calculated from these data were 72 ($\pm 0.80\%$) and 63 ($\pm 1.28\%$) for IM and SRG plots respectively.

Figure 5.14 compares daily rainfall data with mean soil WFPS for the three plots under each management on each sampling occasion at both sites in 2012. Mean % WFPS varied between 53.6 and 87.3% in the SRG plots and between 40 and 79% in the IM plots. Of the 18 sampling occasions, WFPS was 60% or higher on 17 and 12 occasions at the SRG and IM plots respectively. The % WFPS generally increased in response to rainfall and responded in a similar way at both the IM and SRG plots, except for a tendency for mean WFPS in the IM plots to decrease more rapidly than in the SRG plots following periods of low rainfall.

On every occasion the mean WFPS was lower in the IM plots than the SRG plots and on nine of the 18 occasions this difference was found to be statistically significant ($P \leq 0.05$). The mean (n=18) difference between the average WFPS of the IM and SRG plots across all the sampling occasions was 12.04 ($\pm 5.77\%$), a significant difference in mean WFPS between the three plot pairs was only found on one occasion ($P \leq 0.05$).

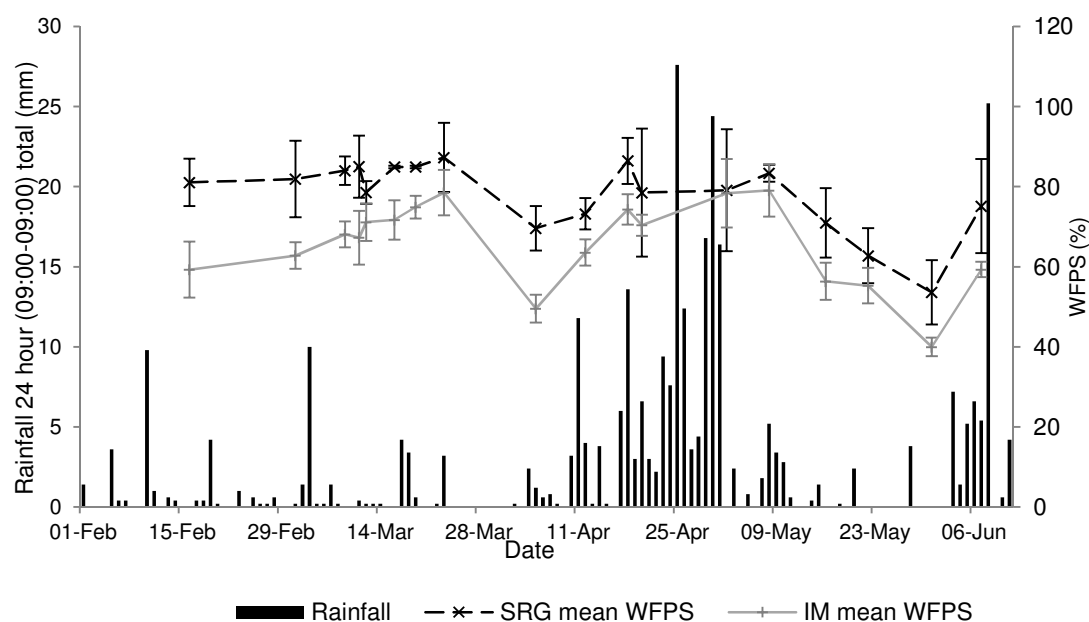


Figure 5.14 2012 daily rainfall totals for 24 hour period (09:00-09:00) and mean ($n=3$) WFPS at SRG and IM sites. Error bars showing ± 1 SD

5.4.2 Soil available N (2012)

The mean soil NO_3^- -N concentration was greater than the mean NH_4^+ -N for all plots except for in May 2012 (Figure 5.15). The greatest mean ($n=3$) NO_3^- -N concentration for each site was found in samples taken on 23 March, which was 14 days after the first fertiliser application, when NO_3^- -N concentrations were $59.3 (\pm 16.8 \text{ mg N kg}^{-1})$ of dry soil) and $277 (\pm 39.5 \text{ mg N kg}^{-1})$ of dry soil) at the SRG and IM plots respectively.

Soil NH_4^+ -N concentrations were similar at both sites on all sample occasions and were always $< 9 \text{ mg N kg}^{-1}$ of dry soil, apart from on 16 May (29 days after the second fertiliser application), when the mean concentrations in the SRG and IM plots were $32.7 (\pm 12.3 \text{ mg N kg}^{-1})$ of dry soil) and $124.8 (\pm 7.14 \text{ mg N kg}^{-1})$ of dry soil) respectively.

On the five sampling dates in March and April, the mean soil NO_3^- -N concentration in the IM plots was significantly greater than in the SRG plots, but on the later sampling dates in May and June there was no significant difference in NO_3^- -N

concentration between the two sites. The only sampling date for which there was a significant difference in mean $\text{NH}_4^+\text{-N}$ concentration between the two sites was the 16 May, when the mean concentration in the IM plots was greater than in the SRG plots

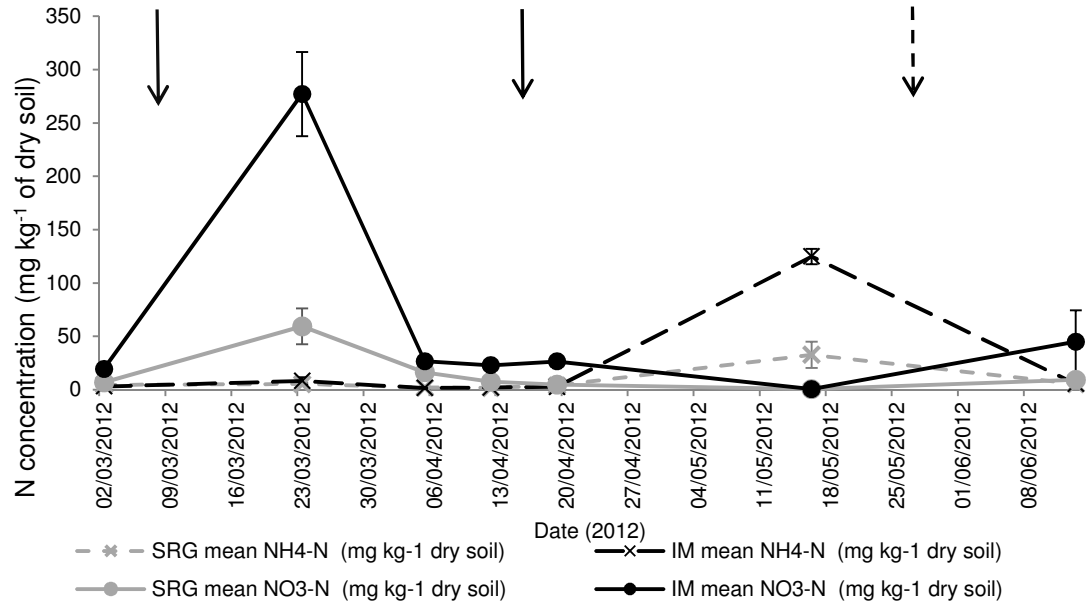


Figure 5.15 Mean ($n=3$) N available as $\text{NO}_3^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ at IM and SRG sites in 2012, showing dates of fertiliser application (solid arrows) and grass cutting (dashed arrows) at IM plots. At the first fertiliser application 80 kg N ha⁻¹ and at the second application 39 kg N ha⁻¹ of ammonium nitrate fertiliser were applied. Error bars show ± 1 SD

5.5 N_2O fluxes

5.5.1 Analysis of N_2O flux data

Data from N_2O flux measurements were analysed to identify any effect of management (IM / SRG) on soil N_2O flux on any given sampling occasion and whether any effects were found consistently across multiple sampling occasions. In 2010 / 2011 the N_2O sampling occasions were not sufficiently frequent or evenly spaced in time to carry out a time series analysis, so a separate two factor ANOVA without replication was conducted in Microsoft Excel on the mean N_2O flux from each plot for each sampling occasion. Plots were categorized according to site (IM or SRG) and plot pair number (1-4). Regular sampling in 2012 allowed a time series analysis to be undertaken on the N_2O flux data. A repeated-measures ANOVA was carried out on the mean N_2O fluxes from each plot in the IM field and the SRG. The

analysis was carried out using GENSTAT14 software with a Greenhouse-Geisser correction factor. The repeated measured ANOVA on the N₂O flux data found no significant interaction between the effect of sampling date and land management on N₂O flux. A separate two factor ANOVA, as used for analysis of the 2010 / 2011 data was then carried out for each sampling occasion, using GENSTAT14 to identify whether there was any significant relationship between field management and N₂O flux at any one time point and allow comparison with the results from analysis of the 2010 / 2011 data in the discussion. Regression analysis was also conducted in GENSTAT14 to identify the relationship between soil temperature and WFPS at the time of sampling and N₂O flux. Separate linear regression models were fitted to the data from each sampling date, as it was considered that changes in other soil variables, including available soil N concentration, over time would prevent a constant relationship between the covariates (soil temperature and WFPS) and the response variable (N₂O flux). For each sampling date the optimum regression model containing neither, one, or both of the covariates was fitted to the mean N₂O flux values for the six plots.

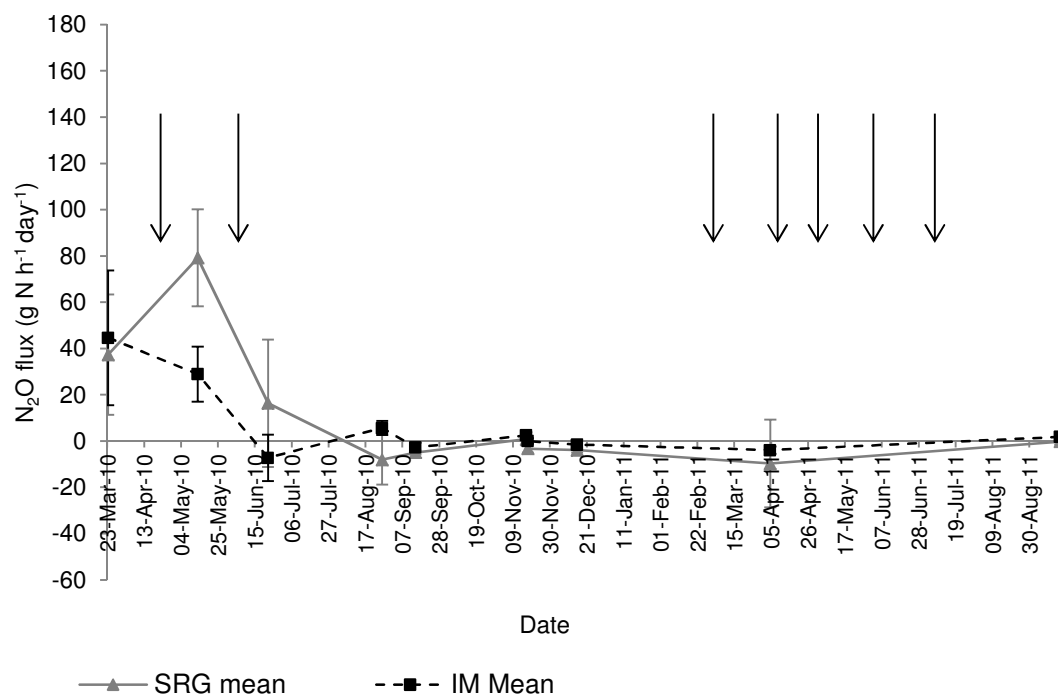
5.5.2 Differences in N₂O flux between IM and SRG plots

In general the N₂O fluxes measured in 2010 and 2011 were small, for both land management types. The only fluxes > 5 g N ha⁻¹ day⁻¹ were recorded during the growing season (March-August) in 2010 (Figure 5.16). The greatest measured N₂O fluxes from both plot types were on 13 May 2010. The IM plots had received 80 kg N ha⁻¹ ammonium nitrate fertiliser 20 days prior to the peak N₂O flux (Figure 5.17), however the SRG plots did not receive any fertiliser. On 13 May 2010 the mean N₂O flux from the SRG plots was 108 (±20.0 g N ha⁻¹ day⁻¹), which was significantly greater (F(1,3)=195.5, P=0.0008) than the mean flux from the IM plots of 47.3 (±12.3 g N ha⁻¹ day⁻¹) (Table 5.4). The only other date on which there was a statistically significant difference in measured N₂O flux between the IM field and the SRG in the 2010-2011 period was 16 September 2011, when the mean flux from the IM plots, 2.83 (±1.95 g N ha⁻¹ day⁻¹) was significantly greater (F(1,3)=0.2, P=0.024), than the mean flux from the SRG plot, 0.198 (±1.06 g N ha⁻¹ day⁻¹). Occasional negative fluxes were observed at some plots, indicating net soil N₂O influx. The

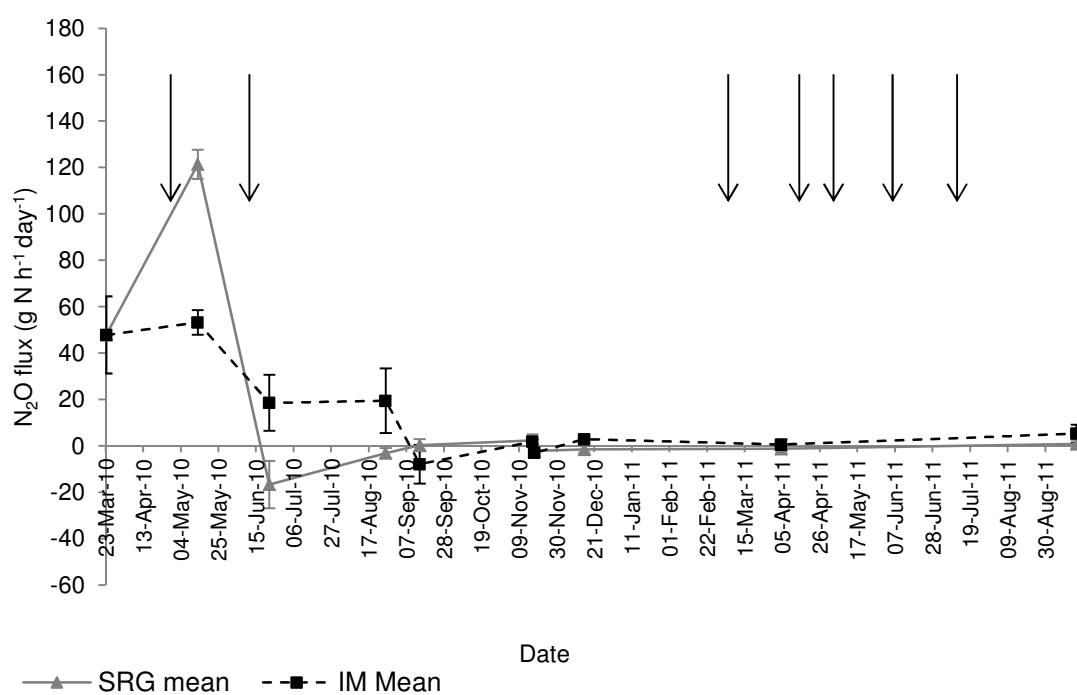
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largest N₂O influx was recorded at SRG plot 2 on 22 June 2010, where the mean (n=4) N₂O flux was -16.7 (\pm 10.2 g N ha⁻¹ day⁻¹) (Figure 5.16b).

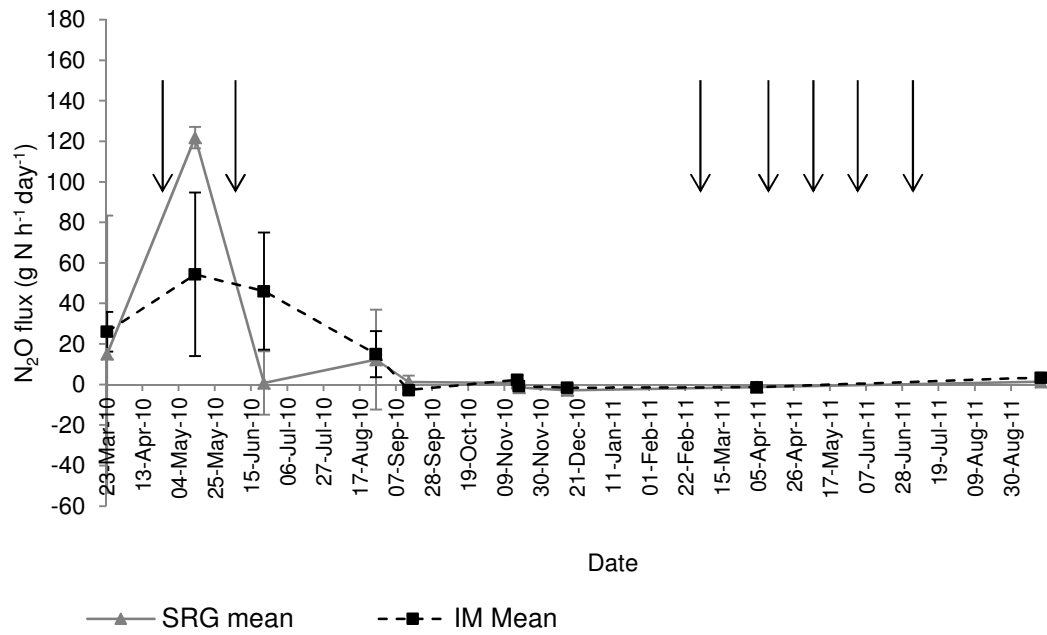
a)



b)



c)



d)

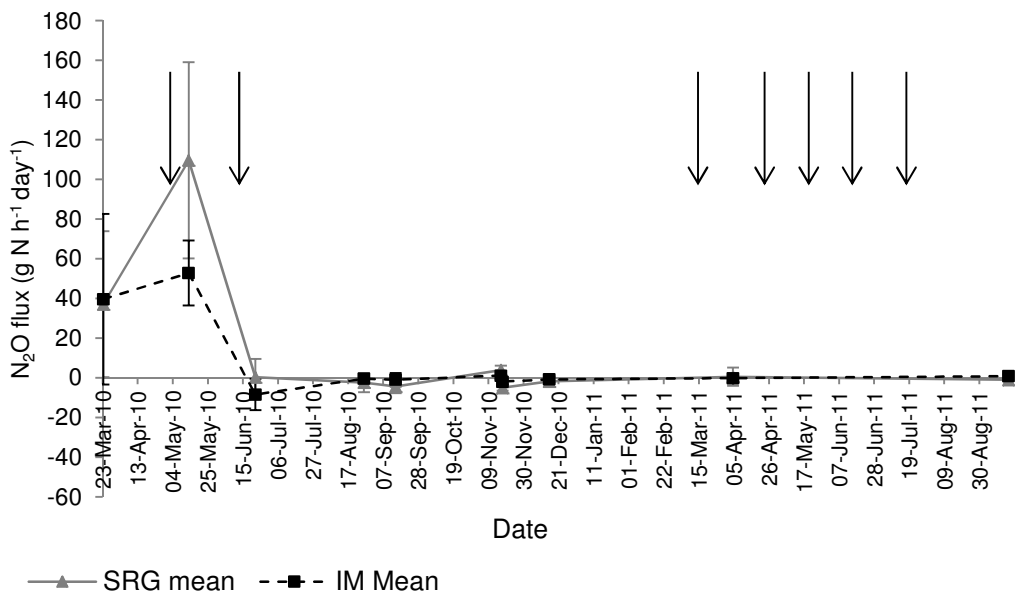


Figure 5.16 Mean ($n=4$) N_2O flux from each of the four paired IM and SRG plots in 2010 and 2011 a) plot pair 1; b) plot pair 2; c) plot pair 3; d) plot pair 4. Error bars show $\pm 1\text{SD}$. Arrows show dates of ammonium nitrate fertiliser application, of 39 kg N ha^{-1} for the 1st four applications followed applications of 34, 39, 50, kg N ha^{-1} respectively for the subsequent three applications.

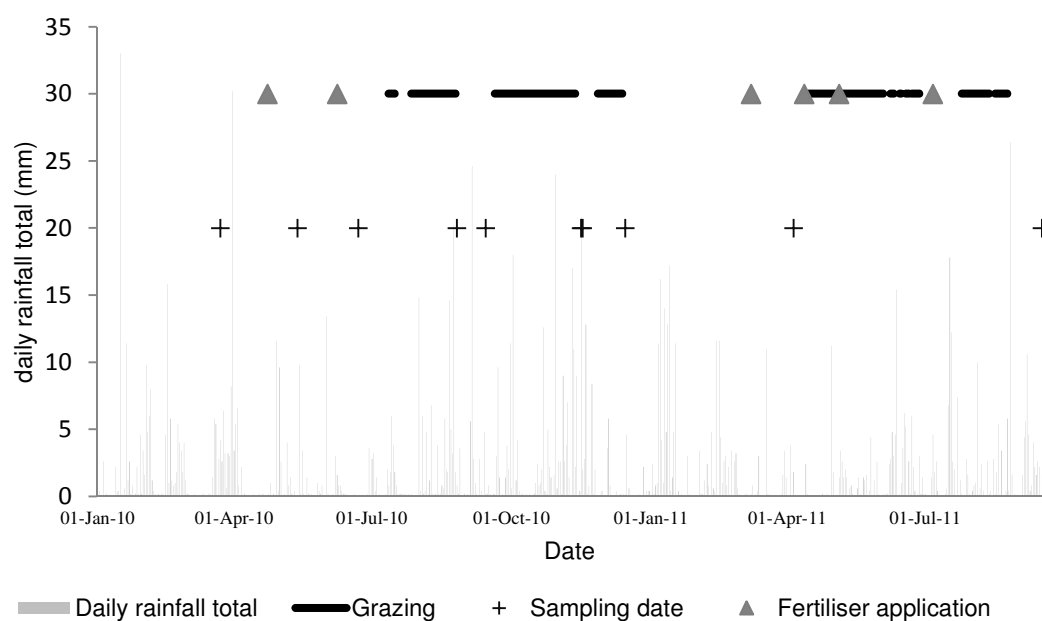


Figure 5.17 Daily rainfall totals for 2010 showing sampling dates for 2010/2011, and grazing/fertiliser regime for IM site. Fertiliser applied on all occasions was ammonium nitrate, quantity of fertiliser applied ranged from 34 - 50 kg N ha⁻¹.

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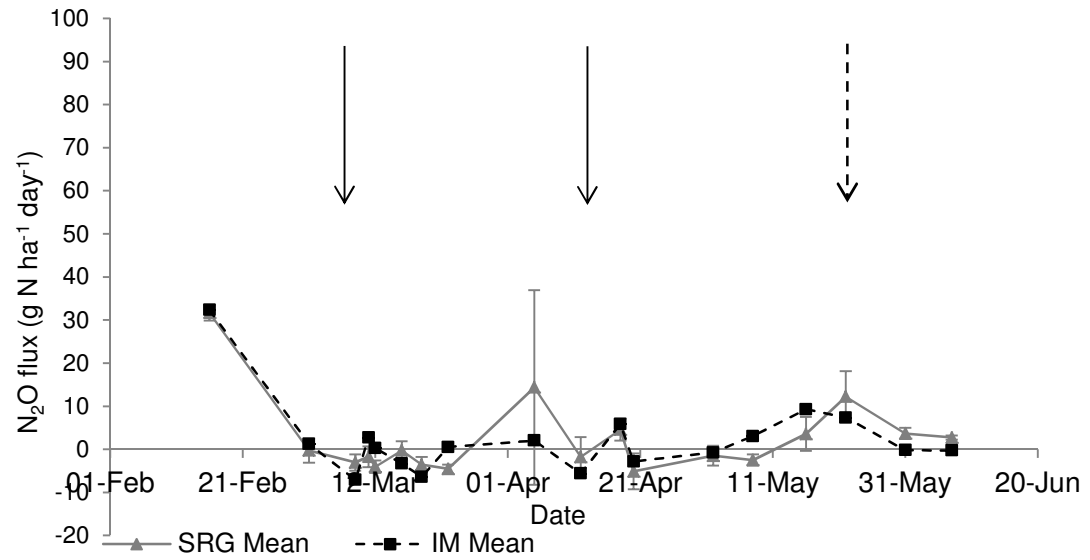
Table 5.4 Mean (n=4) N₂O flux from all IM and SRG plots, along with F statistic and corresponding P value from two factor ANOVA for sampling dates in 2010/2011.

Date (day/month/year)	IM mean (n=4) N ₂ O flux (gNha ⁻¹ day ⁻¹), ± 1SD	SRG mean (n=4) N ₂ O flux (gNha ⁻¹ day ⁻¹) ± 1SD	F(1,3)	P
23-Mar-10	39.5± 9.57	29.7±10.5	8.54	0.061
13-May-10	47.3± 12.3	108±20	196	0.00079
22-Jun-10	12.2± 25.8	0.16±13.5	0.52	0.52
26-Aug-10	9.98±9.07	-0.37±8.79	4.38	0.13
14-Sep-10	-3.6±3.01	-1.98±3.18	0.35	0.59
16-Nov-10	1.91±0.674	1.92±1.47	0.00023	0.99
17-Nov-10	-1.46±1.35	-3.06±1.54	5.42	0.1
15-Dec-10	-0.26±2.12	-2.55±1.08	6.96	0.078
04-Apr-11	-1.25±1.95	-2.96±4.6	1.36	0.33
16-Sep-11	2.83±1.95	0.2±1.06	18.2	0.024

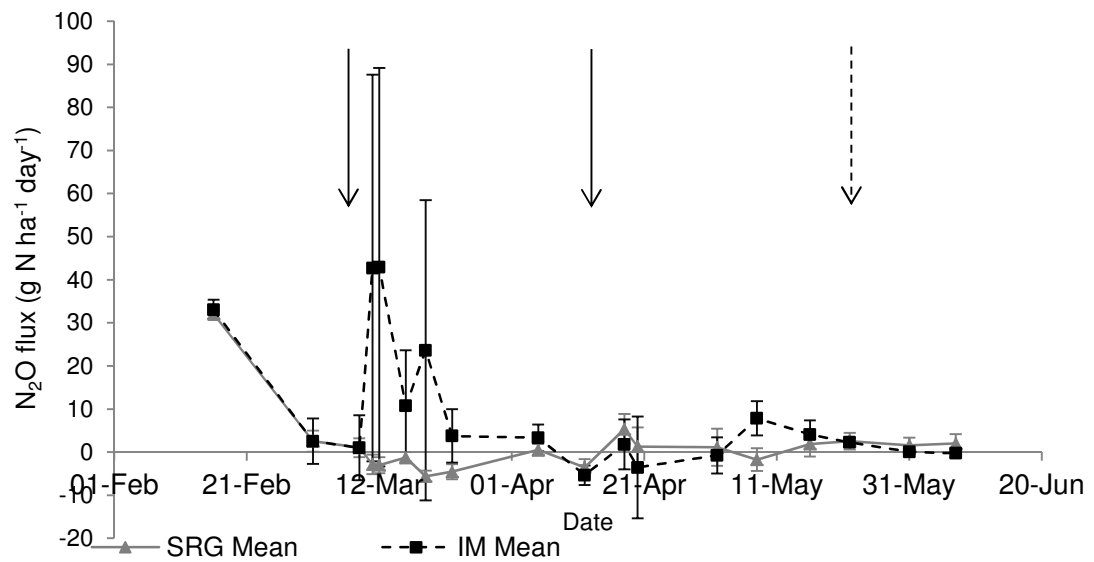
The N₂O fluxes recorded in 2012 were generally low with negative fluxes being recorded on several occasions (Figure 5.18). Fluxes recorded often varied greatly between the four chambers within each plot, giving large standard deviations from the mean. The mean fluxes also varied between plots, although similar patterns were seen over time across the three plots at each site. Mean fluxes over both sites varied significantly with sampling date ($F(17, 68) = 10.07$, $P = 0.018$), but there was no significant interaction between land management and sampling date, ($F(17, 68) = 2.57$, $P = 0.166$). Table 5.5 summarises the results of ANOVAs (two factor) carried out on each sampling date, the only date on which there was a significant difference in mean ($n=3$) N₂O flux between the two management types was 23 March, when the SRG mean of $-3.37 (\pm 1.16 \text{ g N ha}^{-1} \text{ day}^{-1})$ was significantly lower than the IM mean of $2.61 (\pm 1.88 \text{ g N ha}^{-1} \text{ day}^{-1})$, however both these fluxes are very low and do not represent significant peaks in N₂O emissions. The optimised linear regression model fitted to the data from the 23 March included soil temperature as the independent variable and the output from the model was significantly correlated to the measured N₂O fluxes ($F(1, 4) = 15.19$, $P = 0.018$). The simple model explained 73.9% of the variation in the N₂O flux, addition of soil WFPS as independent variable did not improve the model fit. Regression models which included soil temperature as the sole independent variable were also found to be a good fit for the N₂O fluxes measured on 12 March, 16 May and 7 June, ($F(1, 4) = 5.19$, $P = 0.085$); ($F(1, 4) = 5.41$, $P = 0.081$) and ($F(1, 4) = 11.84$, $P = 0.026$) respectively, on all three occasions the correlation between soil temperature and N₂O flux was negative. Regression models that included soil WFPS as the sole independent variable were found to be a good fit for the N₂O fluxes measured on 16 February, 11 March, 19 March 12 April and 8 May, ($F(1, 4) = 12.04$, $P = 0.026$); ($F(1, 4) = 6.03$, $P = 0.07$); ($F(1, 4) = 6.75$, $P = 0.06$); ($F(1, 4) = 6.11$, $P = 0.069$) and ($F(1, 4) = 11.85$, $P = 0.026$) respectively. The correlation between mean N₂O flux and mean soil WFPS across the six plots was negative for all these dates except 12 April.

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a)



b)



c)

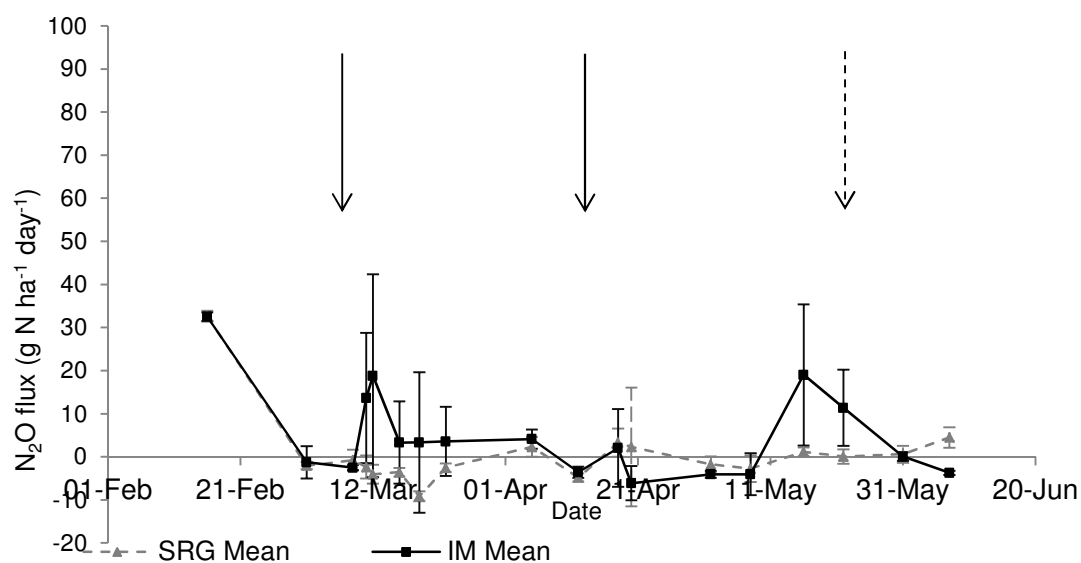


Figure 5.18 Mean flux ($n=4$), error bars ± 1 SD, from SRG and IM sites in 2012, a) Plot 1, b) Plot 2, c) Plot 3. Arrows indicate time of fertiliser application (solid line) and grass cutting (dashed line) at IM plots.

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Table 5.5 Mean (n=3) N₂O flux (g N ha⁻¹ day⁻¹) from IM and SRG sites, along with F statistic and corresponding P value from two factor ANOVA for sampling dates in 2012.

Date (2012)	Mean (n=3) N ₂ O flux (gNha ⁻¹ day ⁻¹), ± 1SD			
	SRG plots	IM plots	F (1,2)	P
16-Feb	32.1±0.48	32.6±0.377	2.59	0.249
02-Mar	0.0847±2.31	0.843±1.94	2.9	0.231
09-Mar	-0.936±2.05	-2.88±4.07	2.75	0.239
11-Mar	-2.32±0.569	17.5±20.7	2.03	0.209
12-Mar	-3.7±0.584	20.7±21.4	4.09	0.181
16-Mar	-1.7±1.70	3.57±7.02	1.42	0.355
19-Mar	-6.11±2.83	6.85±15.3	1.96	0.296
23-Mar	-3.87±1.16	2.61±1.82	45.95	0.021
05-Apr	5.73±7.54	3.16±1.03	0.28	0.651
12-Apr	-3.37±1.53	-4.85±1.11	1.01	0.421
18-Apr	4.42±1.03	3.23±2.29	0.71	0.488
20-Apr	-0.511±4.02	-4.17±1.74	1.35	0.366
02-May	-0.714±1.59	-1.87±1.91	1.49	0.347
08-May	-2.33±0.521	2.28±5.97	2.1	0.284
16-May	2.19±1.24	10.8±7.57	3.28	0.212
22-May	4.95±6.42	7±4.56	0.18	0.712
31-May	1.96±1.59	-0.03±0.148	3.97	0.185
07-Jun	3.08±1.28	-1.41±2.01	5.69	0.14

5.6 Comparisons of DNDC model output and measured N₂O fluxes

5.6.1 Description of the DNDC model

The DNDC model is a process-based model developed by (Li et al., 1992a, 1992b) that simulates the biogeochemical cycling of C and N in soils. The model uses site specific data on ‘ecological drivers’, which include climate, soil properties, vegetation, and anthropogenic activities. These ecological drivers are then fed into three sub-models. The soil climate sub-model predicts soil moisture, temperature and redox potential across the soil profile. The plant growth sub-model uses the input data on the crop, climate, soil properties and management to predict plant growth rates and model the feedback effects of plant growth on soil properties such as moisture, pH, redox potential and C and N concentrations. Finally the decomposition sub-model simulates changes in the size of various soil C and N pools (Li, 2000), the soil C is divided among four major pools, each of which is sub-divided into multiple more or less labile sub-pools (Wang et al., 2012). The three sub-models feed back into one another to determine the combined effects of the ecological drivers on the soil environmental variables. These calculated environmental variables are then fed into a fourth sub-model; the denitrification / nitrification sub-model, which predicts the fluxes of NO and N₂O gasses from the soil. The equations used to drive this process-based model were derived either from empirical data or from the classical laws of physical, chemical and biological processes Li et al. (1992a, 1992b).

The model used for this study was the UK-DNDC model, a version of the DNDC model calibrated according to the crops, soil characteristics, livestock and farming practices in the UK (Brown et al., 2002; Wang et al., 2012). This model version has been optimized and validated for increased accuracy in calculating N₂O fluxes from grazed grassland systems. Nitrous oxide production and consumption, as determined by the model, alters in response to soil redox potential, dissolved organic C and available N concentration; a decline in any of these factors will decrease modelled N₂O fluxes. Changes to the input values for any of the ecological drivers can alter one or more of the factors determining N₂O emissions in the model, and therefore regulate the calculated N₂O fluxes (Wang et al., 2012).

5.6.2 Model inputs and data analysis

Modelled N₂O emissions were compared with field measurements taken from the NW IM and SRG plots during the period of intensive sampling undertaken in 2012. The model was run for two consecutive years, 2011 and 2012. The inputs assumed a perennial grass sward was planted on 1 January 2011 (year1) and the harvest date was set for the end of 2012 (year2), with the field described as fallow in 2012. This method of modelling for one year prior to the year of interest has been found to optimise the model output for N₂O emissions from permanent grassland (Gallejones, 2012). The climate data input files included daily values for total rainfall (cm), minimum temperature (°C) and maximum temperature (°C) as measured at the Burrows AWS NW, described in detail in section 5.1. All management information (Table 5.6 –Table 5.8) was provided by the NW Farm Manager. The methods used for obtaining data by field measurements (Table 5.9) are detailed in Chapter 3. Where input values are not stated, the UK-DNDC default values were used. The total modelled N₂O fluxes over the sampling period were calculated using the area under the curve function in GENSTAT14. This function was also applied to the combined mean flux from all three plots under each management, to give an estimate of the total N₂O flux for the same period from the IM and SRG field based on field measurements.

Table 5.6 Fertiliser applications at the IM plots entered under the management tabs for year1 (2011) and year2 (2012) of the UK-DNDC model,

Date	Fertiliser type	Application mode	Quantity (kg N ha ⁻¹)
08/03/2011	Ammonium Nitrate	Surface	39
12/04/2011	Ammonium Nitrate	Surface	39
05/05/2011	Ammonium Nitrate	Surface	34
06/06/2011	Ammonium Nitrate	Surface	39
07/06/2011	Ammonium Nitrate	Surface	50
09/03/2012	Ammonium Nitrate	Surface	80
17/04/2012	Ammonium Nitrate	Surface	39

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Table 5.7 Details of grazing periods on IM plot in year1 (2011), all grazing carried out without supplementary feeding, with livestock present for 24 hours a day.

Type of Livestock	Date on to field	Date off field	Grazing density (head ha ⁻¹)
Beef cattle	15/04/2011	03/06/2011	6.74
Sheep	08/06/2011	10/06/2011	19.43
Sheep	14/06/2011	15/06/2011	19.43
Beef cattle	18/06/2011	20/06/2011	6.47
Beef cattle	22/06/2011	24/06/2011	6.47
Beef cattle	25/06/2011	27/06/2011	6.47
Beef cattle	25/07/2011	12/08/2011	6.47
Beef cattle	16/08/2011	24/08/2011	6.47
Sheep	21/09/2011	10/10/2011	26.17
Sheep	28/10/2011	09/11/2011	26.94
Sheep	14/11/2011	15/11/2011	26.68
Sheep	15/12/2011	21/12/2011	25.91

Table 5.8 Dates of cutting and cut fraction at both IM and SRG plots in year1 (2011) and year2 (2012), used for input into UK-DNDC model.

Plots	Date cut	Cut fraction
SRG	02/06/2011	0.9
SRG	20/08/2011	0.9
IM	27/05/2012	0.9
SRG	26/07/2012	0.9

Table 5.9 UK-DNDC Input values used for soil and site properties, for both sets of plots (IM and SRG), including details of data source

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Parameter	Input value		Data source
	IM plots	SRG plots	
land-use type	3 moist grassland	3	NA
Soil texture	Clay loam	Clay loam	(Harrod and Hogan, 2008)
Clay fraction	0.4	0.4	(Harrod and Hogan, 2008)
BD	0.72	0.95	Field measurements
Soil pH	5.8	5.8	Field measurements
SOC at surface	0.053 (kgCkg ⁻¹)	0.038	Field measurements
Litter_SOC	0.01667	0.01667	(Li, 2012)
Humads_SOC	0.13258	0.13258	(Li, 2012)
Humus_SOC	0.85075	0.85075	(Li, 2012)
Initial NO ₃ ⁻ -N	22.3 (mgNkg ⁻¹)	6.23	Field measurements
Initial NH ₄ ⁺ -N	26.2 (mgNkg ⁻¹)	16.9	Field measurements
Field capacity	0.7	0.7	(Li, 2012)
Wilting point	0.4	0.4	(Li, 2012)
Slope	5	5	Field measurements
Crop type	12 (perennial grassland)	12	NA
Plant time (month/day)	1/1	1/1	(Gallejones, 2012)
Harvest time	12/31	12/31	(Gallejones, 2012)
Year of harvest	2 (second year)	2	(Li, 2012)
Biomass C/N ratio (leaf and stem)	11.5	12.9	(Gallejones, 2012)

The model output values for the three month period over which field measurements were carried were compared for the two management types to identify whether the

general patterns observed in the measured datasets were shown in the model outputs. To analyse model performance directly the model output values were compared with the field measurements of N₂O flux, soil available N and soil WFPS. Pearson's Product-Moment Correlations coefficient was calculated to determine the correlation between the measured values and the model output value for the sample date for each of the measured soil variables. The significance of the R² value was determined by converting the calculated R² value to a corresponding t value (Equation 13) thus enabling Pr to be calculated based on t having a standard t-distribution .

$$t = \frac{R}{\sqrt{(1-R^2)/(n-2)}} \quad (13)$$

5.6.3 Comparing model output with field measurements of N₂O flux

The model output predicted a total N₂O flux of 0.889 kg N ha⁻¹ from the IM plots for the period 13 February to 13 June 2012, this was 33.7 times greater than total N₂O flux of 0.0264 kg N ha⁻¹ predicted from the SRG plots for the same period (Figure 5.19). The total N₂O flux estimated for the same period from the field measurements, using the area under the curve function in GENSTAT (Section 5.6.2), was 0.487 kg N ha⁻¹ for the IM plots, which was 1.8 times the estimated flux from the SRG (0.270 kg N ha⁻¹).

For the 18 sampling dates in 2012, fluxes calculated from field measurements in the IM plots recorded a total N₂O flux of 0.0981 kg N ha⁻¹, the sum of the model output values for the same 18 dates was 1.79 times greater (0.176 kg N ha⁻¹). Fluxes calculated from field measurements in the SRG plots recorded a total N₂O flux of 0.0290 kg N ha⁻¹, a value 9.18 times greater than the sum of the model output values for the same dates (0.00316 kg N ha⁻¹).

The N₂O flux calculated from field measurements in the IM plots on 16 February (0.0326 kg N ha⁻¹) was 64 times greater than the model output value for the same day (0.000509 kg N ha⁻¹) (**Error! Reference source not found.**). The model output showed a peak in N₂O emissions of 0.0207 kg N ha⁻¹ from the IM plots on 10 March, field measurements on this date recorded a negative N₂O flux (-0.00288 kg N ha⁻¹). However on 12 and 13 March peaks in mean N₂O flux of 0.0197 and 0.0207 kg N ha⁻¹ respectively were recorded at the IM plots. On the sampling dates from 13

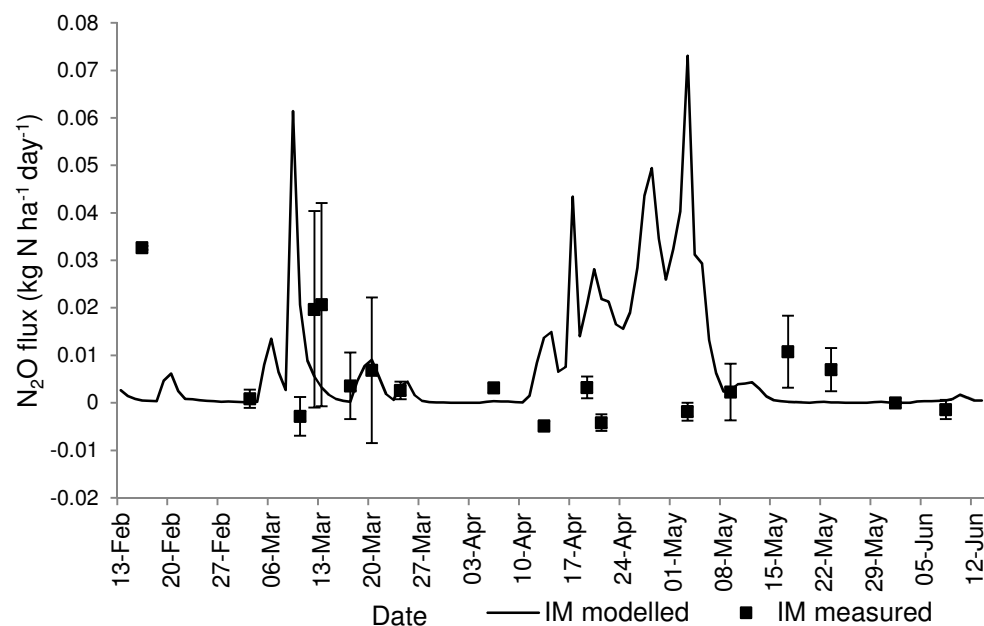
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April to 3 May inclusive and on 17 May, the measured N₂O flux was more than 0.01 kg N ha⁻¹ lower than the model output value for the IM plots. Overall the N₂O fluxes from the UK-DNDC model output for the IM showed no significant correlation with the values obtained from field measurements, ($R^2=0.108$), ($T(16)=0.435$, $P=0.67$) (Figure 5.20a).

The peak in N₂O flux measured in the SRG plots on 16 February (0.0321 kg N ha⁻¹) was 251 times the model output value for the same day (0.000128 kg N ha⁻¹).

Neither the UK-DNDC model output nor the field measurements showed any substantial emissions peaks (>0.04 kg N ha⁻¹ day⁻¹) from the SRG plots, although the overall correlation between the N₂O flux measured in the SRG sites and the model output for the same date was insignificant, ($R^2=0.0788$), ($T(16)=0.316$, $P=0.756$) (Figure 5.20b).

a) IM plots



b) SRG plots

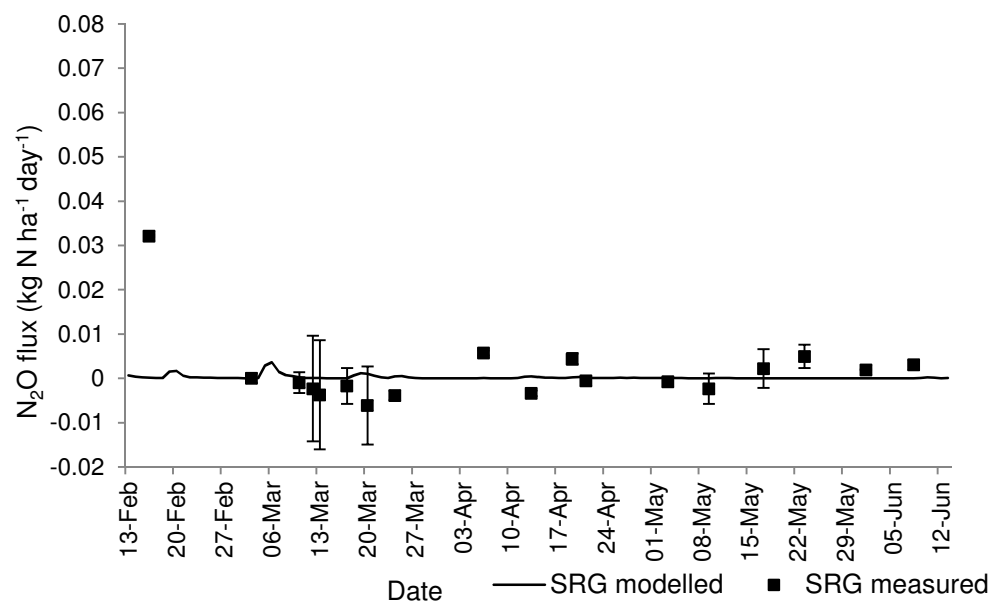
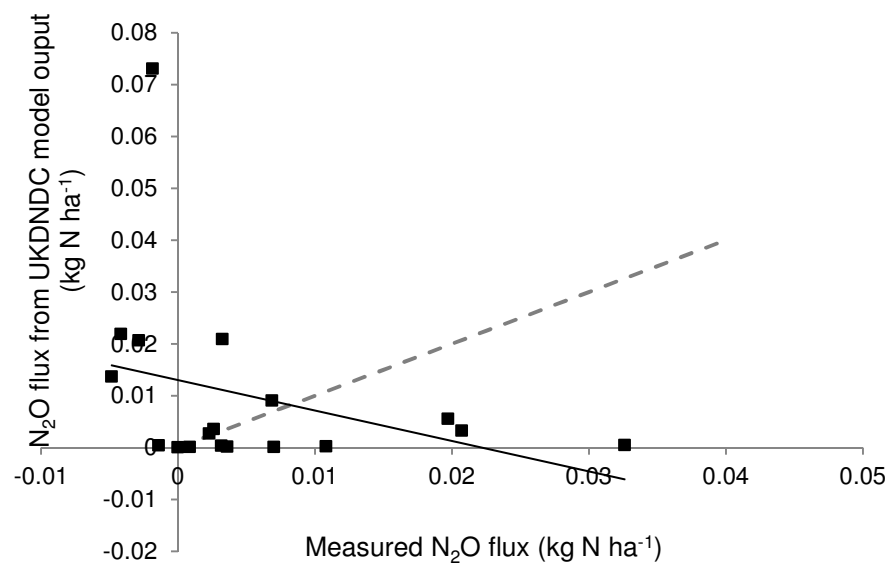


Figure 5.19 UK-DNDC model output for N₂O flux alongside the mean (n=3) N₂O flux measured in the field on each sampling date in 2012, for a) IM and b) SRG plots. Error bars show ± 1 SD.

a) IM plots



b) SRG plots

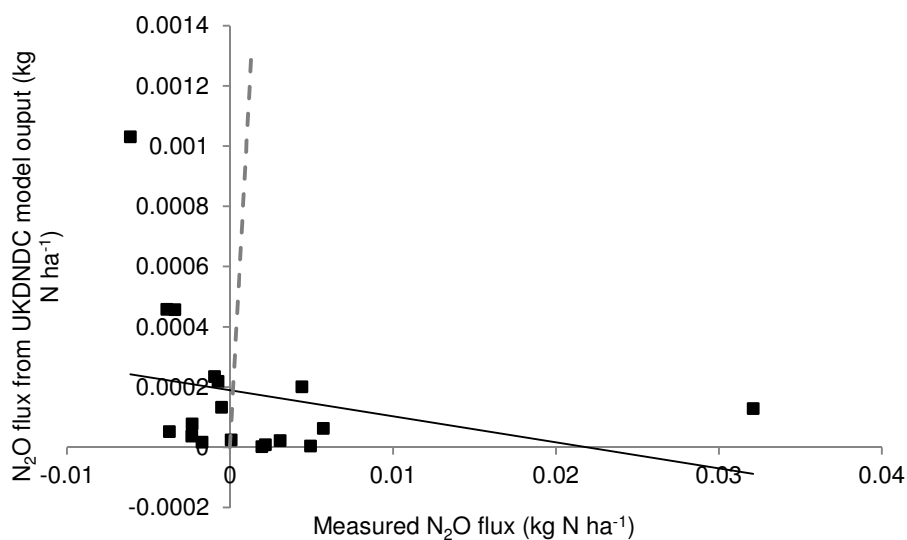


Figure 5.20 N₂O flux from UK-DNDC model output plotted against mean (n=3) N₂O flux measured in the field for the same date in 2012, showing best fit linear trend line fitted using Microsoft word for a) IM plots and b) SRG plots. Dashed line shows x=y.

Table 5.10 Comparison of mean (n=3) measured N₂O flux with the corresponding value from UK-DNDC model output for each sampling date in 2012 for IM and SRG plots.

Date (2012)	IM Plots			SRG plots		
	Mean (n=3)	Modelled	Difference	Mean (n=3)	Modelled	Difference
	N ₂ O flux (kg N ha ⁻¹ day ⁻¹)	N ₂ O flux (kg N ha ⁻¹ day ⁻¹)	(measured- modelled)	N ₂ O flux (kg N ha ⁻¹ day ⁻¹)	N ₂ O flux (kg N ha ⁻¹ day ⁻¹)	(measured- modelled)
16-Feb	0.0326	0.000509	0.0321	0.0321	0.000128	0.0320
03-Mar	0.000843	0.000101	0.000742	0.0000847	0.000024	0.0000607
10-Mar	-0.00288	0.0207	-0.0236	-0.000936	0.000234	-0.00117
12-Mar	0.0197	0.00555	0.0142	-0.00232	0.000077	-0.00240
13-Mar	0.0207	0.00327	0.0174	-0.0037	0.000051	-0.00375
17-Mar	0.00357	0.000186	0.00338	-0.0017	0.000016	-0.00172
20-Mar	0.00685	0.00906	-0.00221	-0.00611	0.00103	-0.00714
24-Mar	0.00261	0.00356	-0.000950	-0.00387	0.000458	-0.00433
06-Apr	0.00316	0.000356	0.00280	0.00573	0.000062	0.00567
13-Apr	-0.00485	0.0137	-0.0186	-0.00337	0.000456	-0.00383
19-Apr	0.00323	0.0209	-0.0177	0.00442	0.0002	0.00422
21-Apr	-0.00417	0.0219	-0.0261	-0.000511	0.000132	-0.000643
03-May	-0.00187	0.0731	-0.0750	-0.000714	0.000218	-0.000932
09-May	0.00228	0.0027	-0.000420	-0.00233	0.000037	-0.00237
17-May	0.0108	0.00024	0.0106	0.00219	0.000008	0.00218
23-May	0.007	0.000105	0.00690	0.00495	0.000004	0.00495
01-Jun	-0.0000273	0.00006	-0.0000873	0.00196	0.000002	0.00196
08-Jun	-0.00141	0.000462	-0.00187	0.00308	0.000022	0.00306

5.6.1 Comparing model output with field measurements of soil WFPS

Soil samples for calculation of soil WFPS were collected from across the 0-7.5 cm depth range, whereas the UK-DNDC model output gives soil WFPS values separately for 1, 5 and 10 cm. To enable a visual comparison between the modelled values and the values calculated from measured soil samples (measured values; see section 3.6.5), the model output for % WFPS at 1, 5 and 10 cm depth is shown alongside measured values (Figure 5.21). Direct comparisons are then made between the model output at 5 cm depth and measured values (Table 5.11 and Figure 5.22).

From Figure 5.21 and Figure 5.22, it was apparent that the UK-DNDC does not generate soil WFPS values that are $> 2\%$ above the given field capacity (FC), except for at 1cm soil depth where there was slightly more flexibility in this upper limit. In contrast measured soil WFPS values were frequently above the FC value used for the model input, leading to poor correlation between the model output and measured values at higher soil WFPS %. Other users of the UK-DNDC model have had similar problems (Gallejones, 2012) and found that decreasing the depth of the soil water retention layer (WRL) in the model, allows output values to exceed this threshold set by the FC during high rainfall events. Parameter optimisation was conducted, to identify whether using a value, other than the default for the depth of the WRL, would improve the correlation between the model output and the measured WFPS. Changing the WRL depth had little impact on the WFPS at 1 cm. There was a bi-modal response in the model output for WFPS at 5 cm, all values of the WRL ≥ 0.342 (including the default value) gave very similar outputs. No improvement in correlation between measured and modelled WFPS was achieved by selecting a value for the WRL of < 0.342 (See appendix D) so the decision was made to keep the default value of 9.99 cm.

The mean ($n=121$) model output for soil WFPS in the IM plots over the period 13 February to 13 June 2012 was 55.4 and 58% at 1 and 5 cm soil depths respectively. The mean model output for soil WFPS in the SRG plots over the same period was 54.3 and 63.6% at 1 and 5 cm depths respectively. The greater mean WFPS at 5 cm depth in the SRG plots compared to the IM plots over the period is consistent with

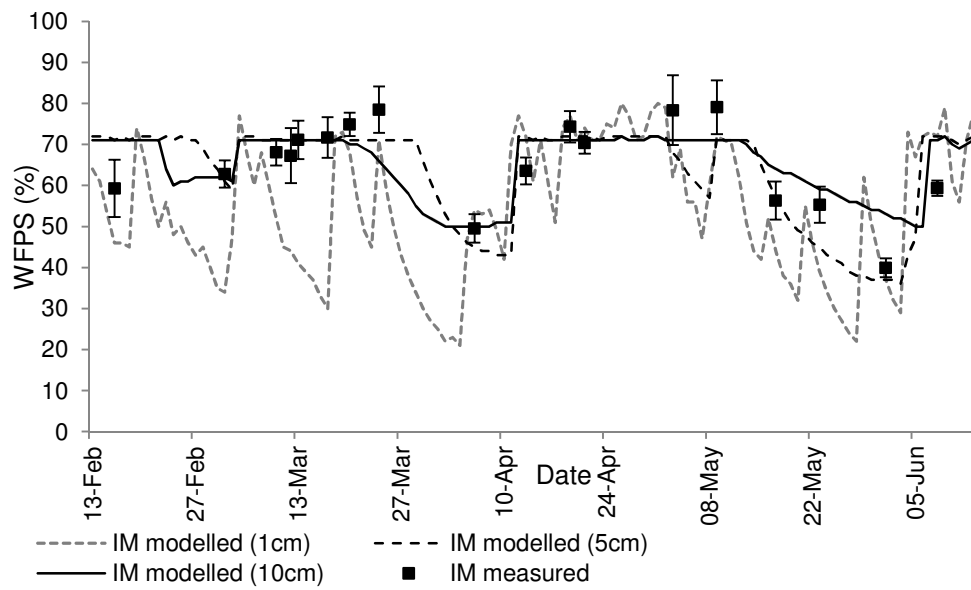
the field measurements, which found that soil WFPS was consistently higher in the SRG plots.

The field measurements of soil WFPS and corresponding UK-DNDC model output values for soil WFPS at 5 cm depth were significantly correlated for the IM ($R^2=0.667$), ($T(16)=3.58$, $P=0.0025$) and SRG plots ($R^2=0.593$), ($T(16)=2.95$, $P=0.00949$).

Over the 18 sampling dates the mean ($n=18$) measured and model output values for soil WFPS in the IM plots were 65.5 and 65% respectively. On average the modelled soil WFPS for a given date at the IM plots differed from the measured soil WFPS by 8.16% of the measured value, the model output was within 5% of the measured value on seven occasions and within 10% on 13 occasions. The greatest % difference between the model output and field measurement of soil WFPS for the IM plots was on 8 June when the model output (72%) differed from measured value (59.3%) by 21.4% of the measured WFPS.

The mean ($n=18$) measured and model output values for soil WFPS over the 18 sampling occasions were 77.8 and 63.7% respectively. On average the modelled soil WFPS for a given date differed from the measured soil WFPS by 18.4% of the measured value. The model output was within 5% of the measured value on 1 occasion, within 10% on four occasions and differed more than 20% on five occasions.

a) IM plots



b) SRG plots

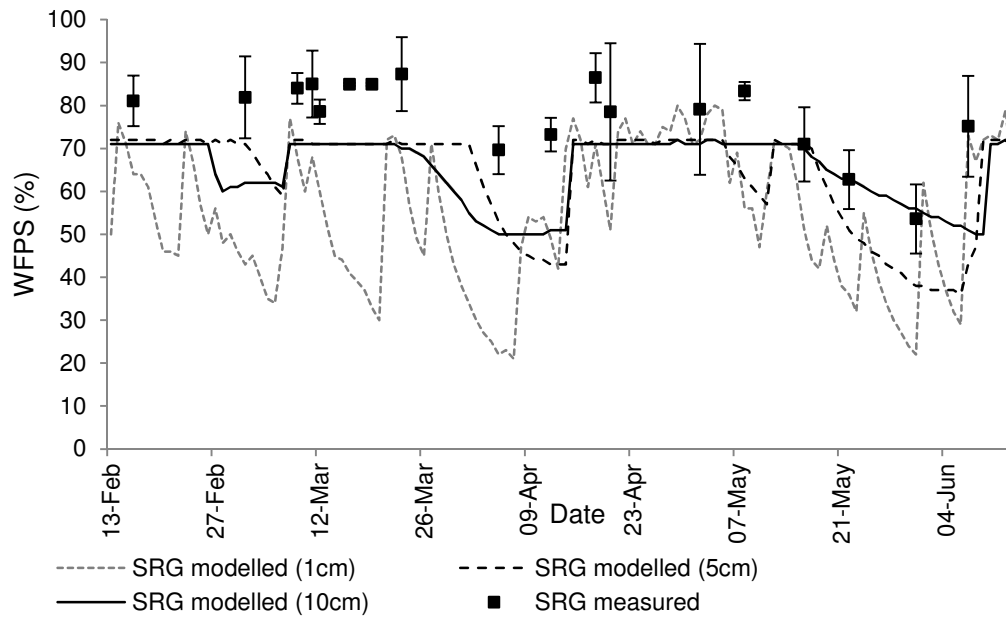
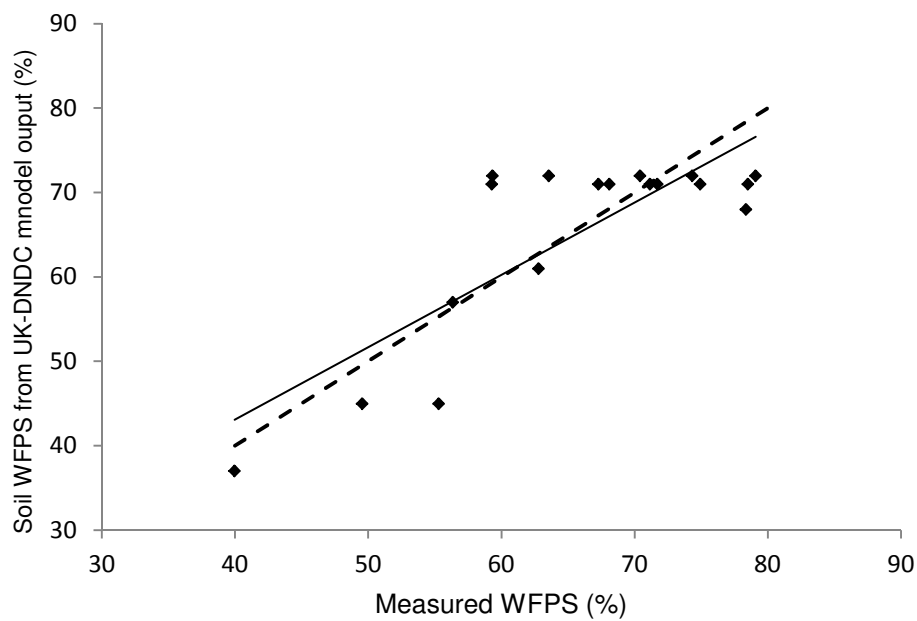


Figure 5.21 UK-DNDC model output for % WFPS at 1, 5 and 10 cm depth with mean ($n=3$) alongside measured soil WFPS from the analysis of samples taken in the field from across the 0 - 7.5 cm depth range for each sampling date in 2012, for a) IM and b) SRG plots. Error bars show ± 1 SD.

a) IM plots



b) SRG plots

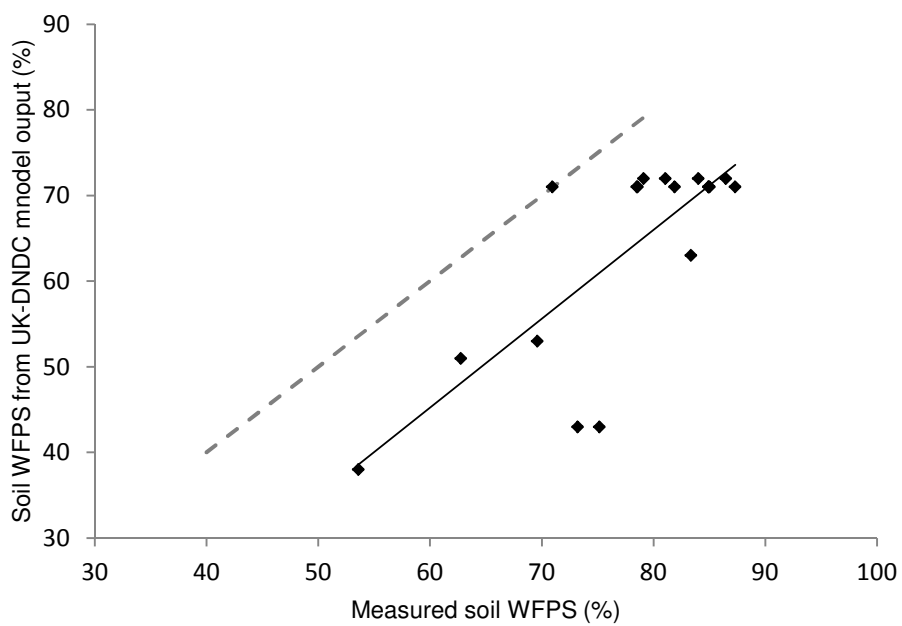


Figure 5.22 UK-DNDC model output for soil WFPS at 5 cm depth plotted against mean (n=3) measured soil WFPS of soil samples taken across the 0-7.5 cm depth range on the same date for a) IM and b) SRG plots. Dashed line shows $x=y$.

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Table 5.11 Comparison of mean (n=3) measured soil WFPS with the corresponding value from UK-DNDC model output for each sampling date in 2012 for IM and SRG plots.

Date (2012)	IM Plots				SRG plots			
	Mean (n=3) WFPS (%)	Modelled WFPS (%) at 5 cm depth	Difference (measured- modelled)	% Difference (% of measured value)	Mean (n=3) measured WFPS (%)	Modelled WFPS (%) at 5 cm depth	Difference (measured- modelled)	% Difference (% of measured value)
16-Feb	59.3	71	-11.7	-19.8	81.1	72	9.1	11.2
03-Mar	62.8	61	1.8	2.85	81.9	71	10.9	13.3
10-Mar	68.1	71	-2.9	-4.27	84.0	72	12.0	14.3
12-Mar	67.3	71	-3.7	-5.53	85.0	71	14.0	16.5
13-Mar	71.1	71	0.1	0.197	78.6	71	7.6	9.63
17-Mar	71.7	71	0.7	0.962	84.9	71	13.9	16.4
20-Mar	74.9	71	3.9	5.23	84.9	71	13.9	16.4
24-Mar	78.5	71	7.5	9.57	87.3	71	16.3	18.7
06-Apr	49.5	45	4.5	9.16	69.6	53	16.6	23.9
13-Apr	63.6	72	-8.4	-13.3	73.2	43	30.2	41.3
19-Apr	74.3	72	2.3	3.12	86.5	72	14.5	16.7
21-Apr	70.4	72	-1.6	-2.27	78.5	71	7.5	9.55

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03-May	78.4	68	10.4	13.2	79.1	72	7.1	8.99
09-May	79.1	72	7.1	8.95	83.3	63	20.3	24.4
17-May	56.4	57	-0.6	-1.15	70.9	71	-0.1	-0.0751
23-May	55.3	45	10.3	18.6	62.8	51	11.8	18.7
01-Jun	40.0	37	3.0	7.41	53.6	38	15.6	29.1
08-Jun	59.3	72	-12.7	-21.4	75.1	43	32.1	42.8

5.6.1 Comparing model output with field measurements of available soil N concentration

The mean ($n=121$) NO_3^- -N concentration predicted by the model for the IM plots over the period 13 February to 13 June 2012 ($20.2 \text{ kg N ha}^{-1}$) was over 130 times that predicted for the SRG plots ($0.155 \text{ kg N ha}^{-1}$) (Figure 5.23). The field measurements also showed higher soil NO_3^- -N concentrations in the IM plots during the first part of the sampling period, although the differences were much less than those predicted by the model (Section 5.4.2).

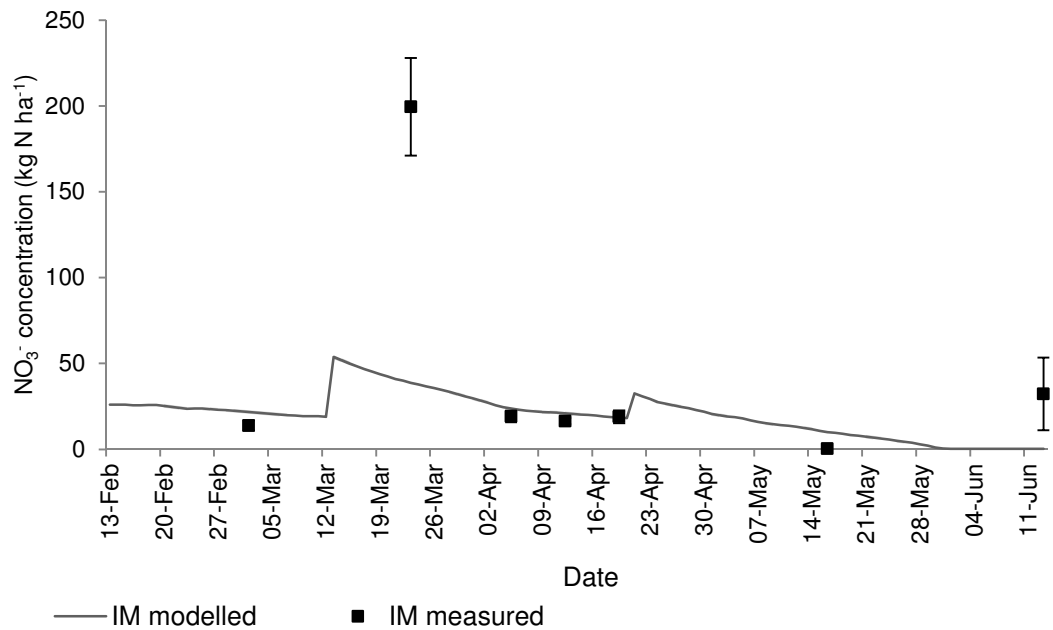
Across the seven sampling occasions the mean ($n=7$) measured soil NO_3^- -N concentration at the IM plots ($43.8 \text{ kg N ha}^{-1}$) was 2.28 times the mean model output ($19.2 \text{ kg N ha}^{-1}$) for the same seven days. The correlation between the model output and mean measured soil NO_3^- -N concentration at the IM plots was weak and insignificant ($R^2=0.475$, $T(5)=1.21$, $P=0.281$) (Figure 5.24). The greatest % difference between the model output and measured soil NO_3^- -N concentration in the IM plots was on 16 May, when the model output (10 kg N ha^{-1}) differed from the measured soil NO_3^- -N concentration ($0.347 \text{ kg N ha}^{-1}$) by 2780% of the measured value (Table 5.12). The best correlation was on 19 April where the model output differed from the measured value by 2.5%. On three occasions, 2 March, 23 March and 13 June, the model output differed from the measured value by $> 50\%$.

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Table 5.12 Comparison of mean (n=3) measured soil NO₃⁻-N concentration with the corresponding value from UK-DNDC model output for each sampling date in 2012 for IM and SRG plots.

	IM Plots				SRG plots		
	Mean (n=3) measured NO ₃ ⁻ -N conc. (kg N ha ⁻¹)	Modelled NO ₃ ⁻ -N conc. (kg N ha ⁻¹)	Difference (measured- modelled)	% Difference (as % of measured value)	Mean (n=3) measured NO ₃ ⁻ -N conc. (kg N ha ⁻¹)	Modelled NO ₃ ⁻ - N concentration (kg N ha ⁻¹)	Difference (measured- modelled)
Date (2012)							
02-Mar	13.9	21.8	-7.9	-56.8	6.45	0.38	6.07
23-Mar	199	38.8	160.2	80.5	56.4	0.05	56.35
05-Apr	19.2	23.7	-4.6	-23.8	15.4	0	15.4
12-Apr	16.4	21.1	-4.7	-28.3	6.92	0	6.92
19-Apr	19	18.5	0.5	2.5	4.49	0	4.49
16-May	0.347	10	-9.7	-2780	0.418	0	0.418
13-Jun	32.4	0.28	32.1	99.1	8.73	0	8.73

a) IM plots



b) SRG plots

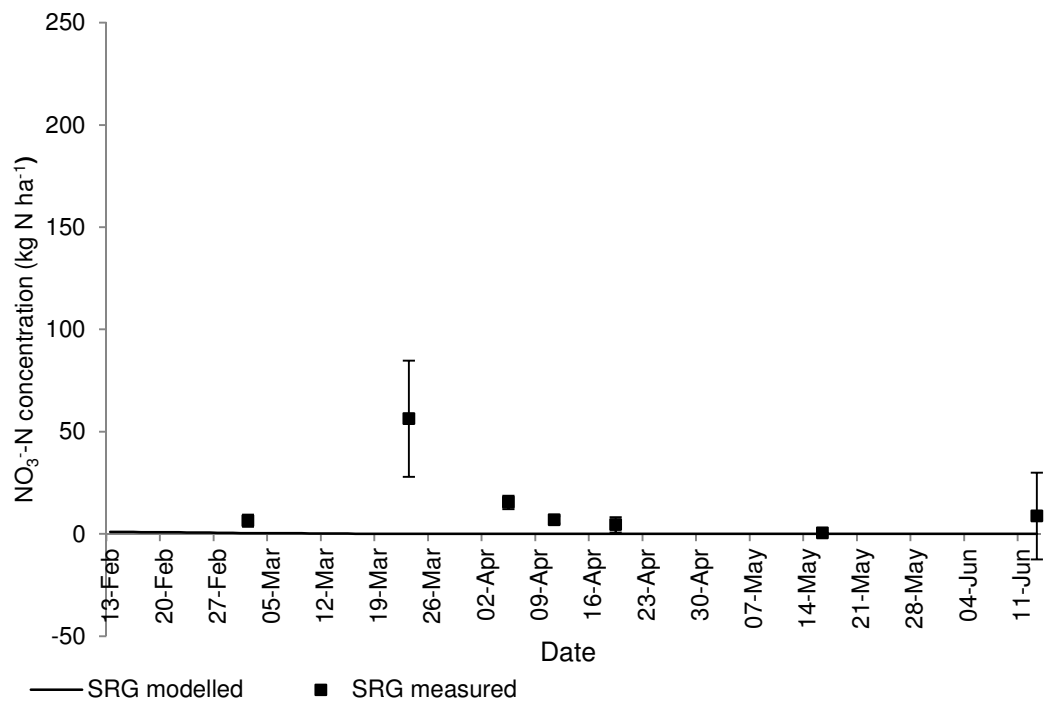
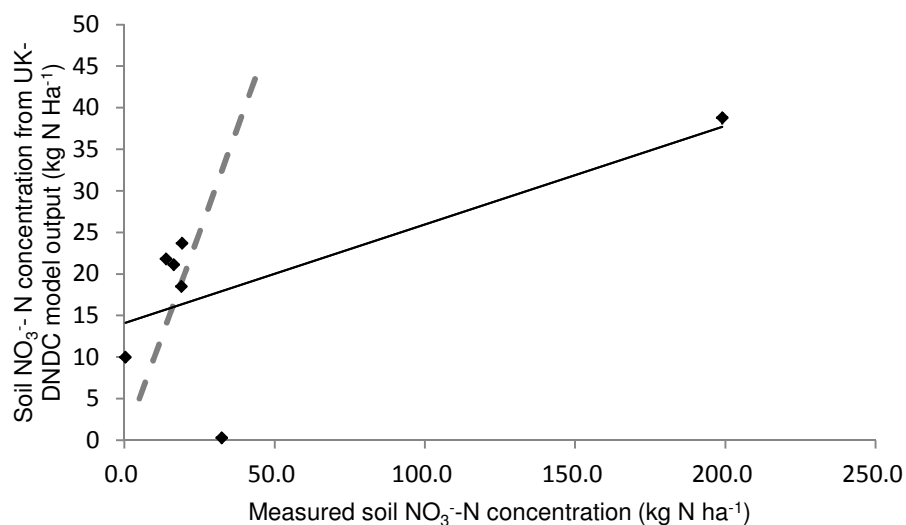


Figure 5.23 UK-DNDC model output for soil NO_3^- -N concentration over the 0-10 cm depth range with alongside mean ($n=3$) measured NO_3^- -N concentration in soil samples collected from across the 0-7.5 cm depth range for each sampling date in 2012, for a) IM and b) SRG plots. Error bars show $\pm 1\text{SD}$.

a) IM plots



b) SRG plots

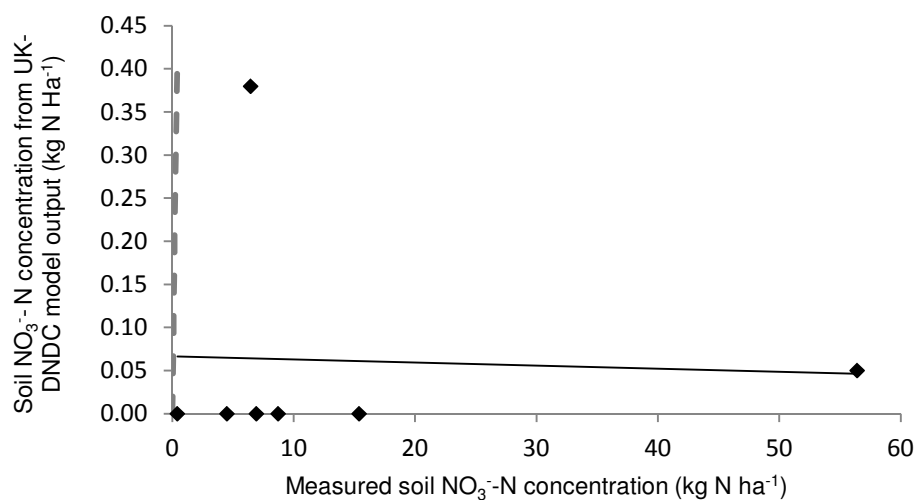


Figure 5.24 Soil NO_3^- -N concentration at 0-10 cm soil depth from UK-DNDC model output plotted against mean ($n=3$) measured NO_3^- -N concentration at 0-7.5 cm depth for the same date in 2012, showing best fit linear trend line fitted using Microsoft excel for a) IM plots and b) SRG plots. Dashed lines show $x=y$.

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The mean ($n=7$) measured soil NO_3^- -N concentration at the SRG plots ($14.1 \text{ kg N ha}^{-1}$) was 235 times greater than the mean model output ($0.06 \text{ kg N ha}^{-1}$) for the same seven days. On all seven dates the model output was lower than the measured soil NO_3^- -N concentration. There was a slight negative correlation between the mean measured soil NO_3^- -N concentrations and the model output for the SRG site, although this was very weak and insignificant ($R^2=0.0024$, $T(5)=0.005$, $P=0.996$).

For all but two of the sampling dates the model output gave a soil NO_3^- -N concentration of 0 kg N ha^{-1} for the SRG plots, the lowest measured soil NO_3^- -N concentration was $0.418 \text{ kg N ha}^{-1}$ on 19 April. On the other six sampling occasions the soil NO_3^- -N concentration was $> 4 \text{ kg N ha}^{-1}$. The largest difference between the measured soil NO_3^- -N concentration and the model output was on 23 March when the model output concentration was $0.05 \text{ kg N ha}^{-1}$ compared to a measured value of $56.4 \text{ kg N ha}^{-1}$.

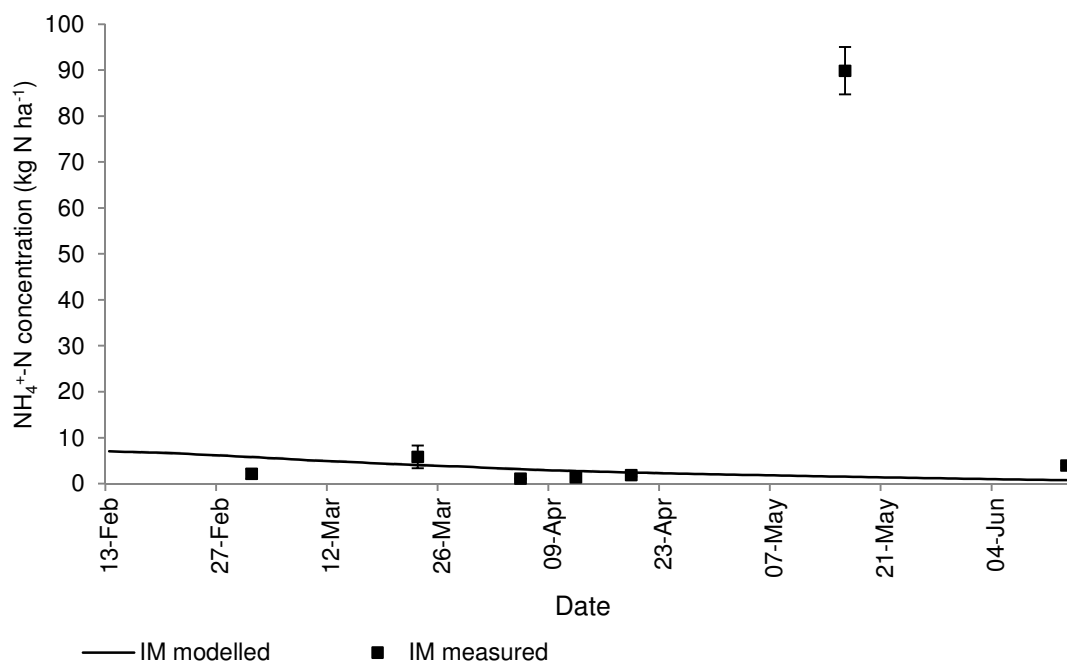
The mean ($n=121$) NH_4^+ -N concentration predicted by the model for the IM plots over the period 13 February to 13 June 2012 ($3.22 \text{ kg N ha}^{-1}$) was 15.5% lower than that predicted for the SRG plots ($3.81 \text{ kg N ha}^{-1}$) (Figure 5.25). The field measurements found no significant difference in soil NH_4^+ -N concentration between the IM and SRG plots, except for on one occasion when the NH_4^+ -N concentration was significantly greater in the IM plots.

Across the seven sampling occasions the mean ($n=7$) measured soil NH_4^+ -N concentration in the IM plots ($15.2 \text{ kg N ha}^{-1}$) was 5.21 times greater than the mean model output for the same seven days ($2.92 \text{ kg N ha}^{-1}$). On every occasion the model output differed from the measured soil NH_4^+ -N concentration in the IM plots by $\geq 30\%$ of the measured value. The mean ($n=7$) modulus of the % difference between the measured soil NH_4^+ -N concentration and the model output for the IM plots was 99.8% (Table 5.13). The model output differed from the measured soil NH_4^+ -N concentration in the IM plots by more than 100% on 2 March and 5 April (168 and 193% respectively). The correlation between the mean concentration of soil NH_4^+ -N at the IM plots on the seven sampling dates and the corresponding model output was slightly negative and insignificant ($R^2=0.14$, $T(5)=0.318$, $P=0.763$) (Figure 5.26)

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The mean (n=7) measured soil NH_4^+ -N concentration in the SRG plots ($8.15 \text{ kg N ha}^{-1}$) was 3.27 times the mean (n=7) model output value ($2.49 \text{ kg N ha}^{-1}$) for the same seven days. On the first two sampling dates, 2 March and 23 March, the model output was greater than the measured soil NH_4^+ -N concentration, on the subsequent five sampling dates the measured concentration was greater. The model output differed from the measured soil NH_4^+ -N concentration by $< 10\%$ on one occasion, 23 March, and by $> 50\%$ on the other six occasions. The correlation between mean measured soil NH_4^+ -N concentration and the model output was weakly negative and insignificant ($R^2=0.0515$, $T(5)=0.115$, $P=0.913$).

a) IM plots



b) SRG plots

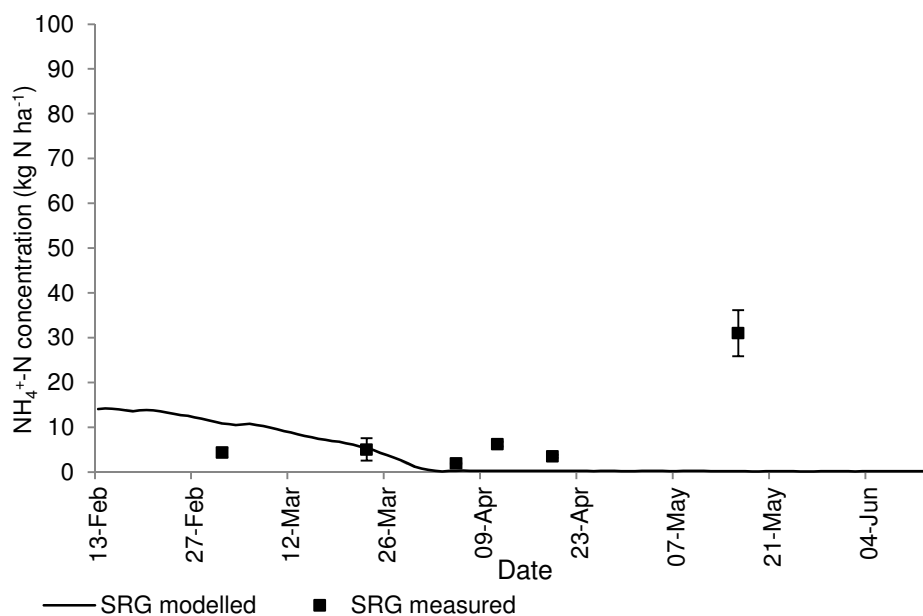
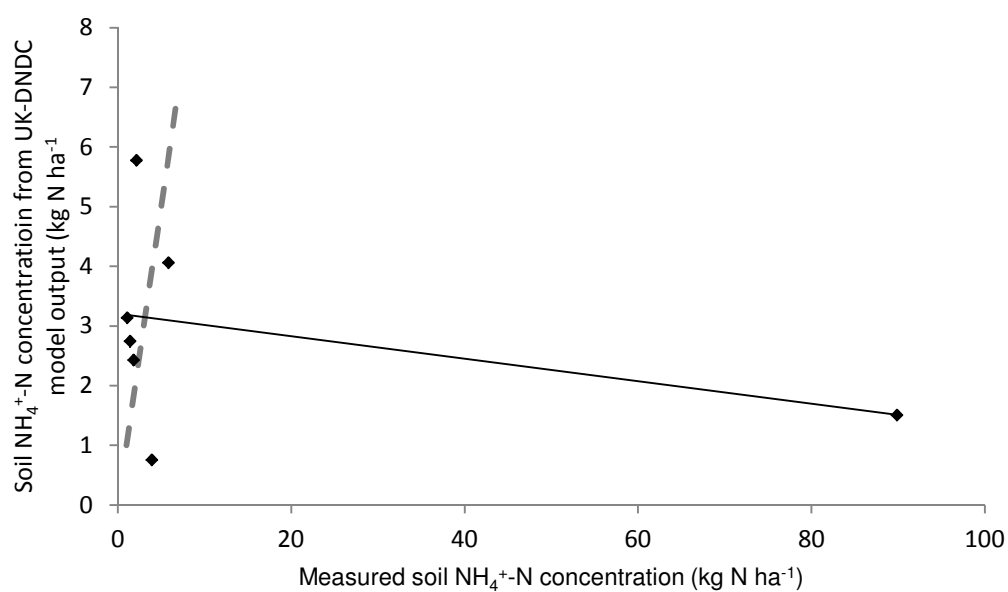


Figure 5.25 UK-DNDC model output for soil NH_4^+ -N concentration over the 0-10 cm depth range alongside with mean ($n=3$) measured NH_4^+ -N concentration in soil samples collected from across the 0-7.5 cm depth range for each sampling date in 2012, for a) IM and b) SRG plots. Error bars show $\pm 1\text{SD}$

a) IM plots



b) SRG plots

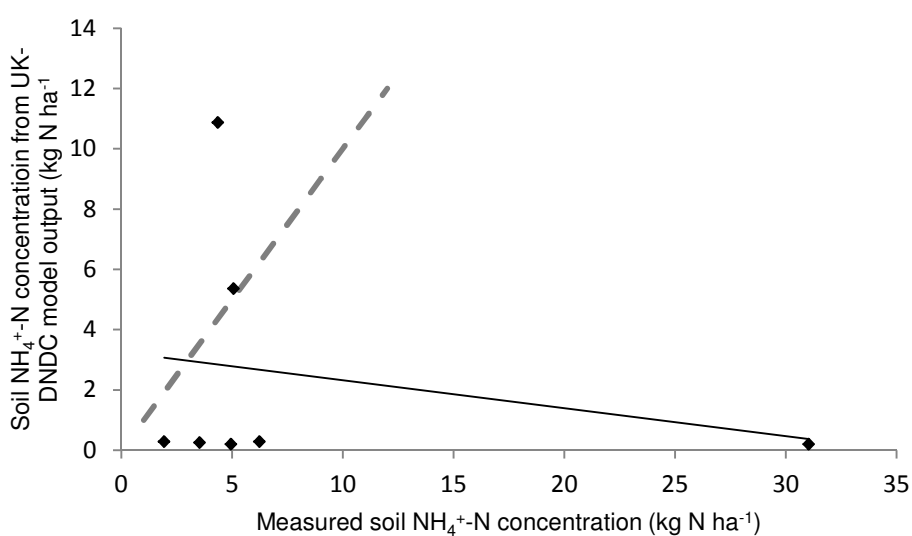


Figure 5.26 Soil NH₄⁺-N concentration at 0-10 cm soil depth from UK-DNDC model output plotted against mean (n=3) measured NH₄⁺-N concentration at 0-7.5 cm depth for the same date in 2012, showing best fit linear trend line fitted using Microsoft excel for a) IM plots and b) SRG plots. Dashed line show x=y.

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Table 5.13 Comparison of mean (n=3) measured soil NH_4^+ -N concentration with the corresponding value from UK-DNDC model output for each sampling date in 2012 for IM and SRG plots

IM Plots					SRG plots			
Date (2012)	Mean (n=3)	Modelled	Difference (measured- modelled)	% Difference (% of measured value)	Mean (n=3)	Modelled	Difference (measured- modelled)	% Difference (% of measured value)
	measured NH_4^+ -N conc. (kg N ha^{-1})	NH_4^+ -N conc. (kg N ha^{-1})			measured NH_4^+ -N conc. (kg N ha^{-1})	NH_4^+ -N conc. (kg N ha^{-1})		
02-Mar	2.16	5.78	-4	-168	4.35	10.9	-6.52	-150
23-Mar	5.84	4.06	2	30	5.05	5.36	-0.306	-6.05
05-Apr	1.07	3.14	-2	-193	1.93	0.28	1.65	85.5
12-Apr	1.40	2.75	-1	-96	6.23	0.28	5.95	95.5
19-Apr	1.84	2.43	-1	-32	3.53	0.25	3.28	92.9
16-May	89.9	1.51	88	98	31	0.19	30.8	99.4
13-Jun	3.93	0.76	3	81	4.94	0.19	4.75	96.2

5.7 Concentrations of N and P compounds in soil water

5.7.1 Analysis of soil water sample data

The limited number of soil water samples collected meant that it was not possible to conduct meaningful statistical analysis on the data. The data that were collected are presented in the following section as preliminary data that could help inform future analysis of soil water samples. The data are from the chemical analysis of soil water samples collected from two water samplers in each plot. A negative pressure was applied to the samplers, which were then allowed to accumulate water over a four day period. The water collected from the two samplers within a plot was combined to ensure sufficient water for the chemical analyses.

5.7.2 Concentrations of total N, total oxidised N, organic N and total P in soil water samples

In January 2011 the total soluble N concentration of the soil water samples from the IM plots was between 2.8 and 9.2 times the concentration in the samples from the SRG plots (Figure 5.27a). The total N concentration of the soil water from the IM plot was also greater than from the paired SRG plot for plot pair 3 in February / March 2011. In contrast, the total N concentration of the soil water samples collected in February / March 2011 from SRG plot 1 was 55% greater than the concentration in the sample from the paired IM plot.

The total oxidised N (TOxN) concentrations (NO_3^- -N + NO_2^- -N) of the soil water samples from the IM plots were on all occasions substantially greater than those of the soil water samples from the paired SRG plot (Figure 5.27b). The TOxN concentration of soil water samples from the IM plots ranged from 5.16 to 47.8 times the TOxN concentration of the soil water samples from the paired SRG plot.

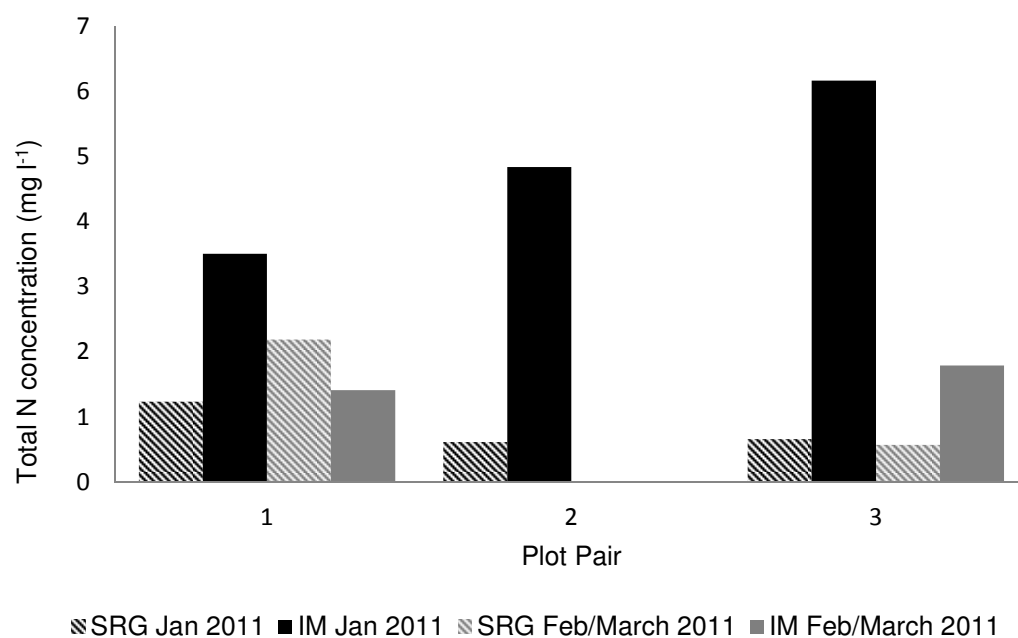
The differences in TOrgN concentration between the soil water samples from the IM and paired SRG plots were smaller than the differences in total N and TOxN (Figure 5.27). In January 2011 the TOrgN concentration in the soil water samples from the IM plot in plot pairs 2 and 3 was 2.59 and 2.28 times the TOrgN concentration of the soil water samples from the paired SRG plot, respectively. Soil TOrgN concentration of the soil water sample collected from the IM plot of plot pair 3 in February / March

2011 was 2.65 times that of the soil water sampled collected from the paired SRG plot. The TOrgN concentrations of the soil water samples collected from the IM and SRG plot of pair 1 in January 2011 were very similar, whilst the TOrgN of the soil water sample collected from the SRG plot 1 in February / March 2011 was 2.24 greater than the TOrgN concentration of the soil water sample collected at the same time from the paired IM plot 1.

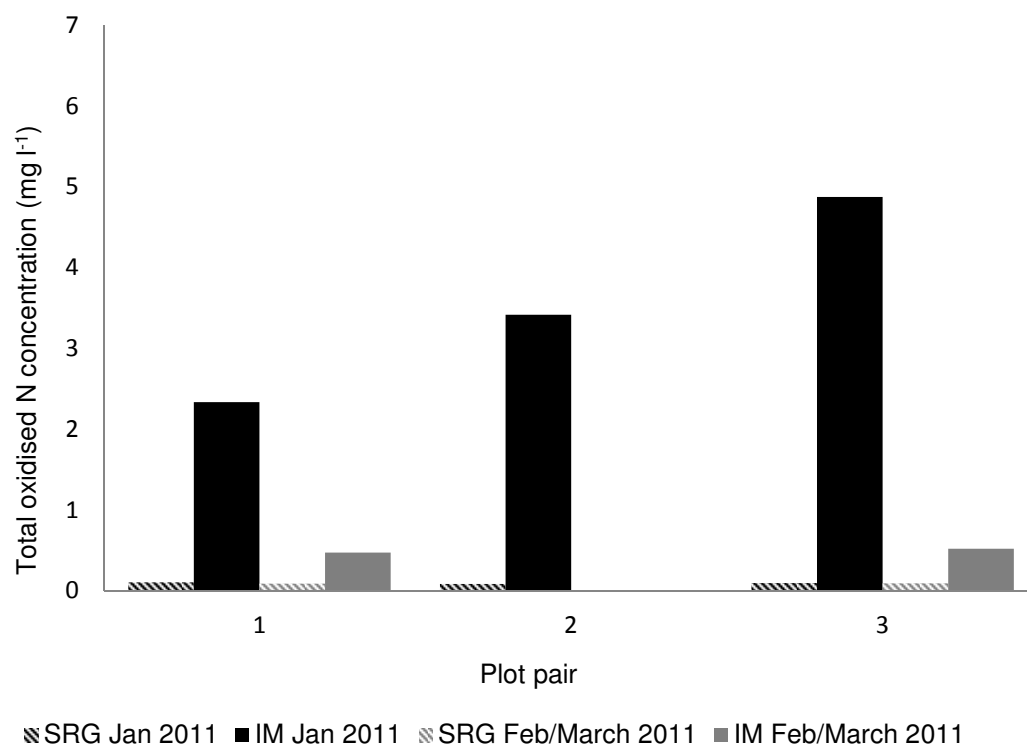
On both occasions and across all plot pairs TOrgN made up a greater % of the total N concentration of the soil water samples from the SRG plot than from the paired IM plot. The differences in the % contribution to total N from TOrgN between paired SRG and IM plots ranged between 12.4 and 63.8% (Figure 5.28).

The total P concentrations were an order of magnitude lower than the total N concentrations of the soil water samples (Figure 5.29). As with the TOrgN concentrations, the total P concentrations of the soil water samples from the IM plots were greater than those of the paired SRG plots in plot pair 2 in January 2011 and plot pair 3 in January and February / March 2011, whilst in plot pair 1, the total P concentrations of the soil water samples from the IM and SRG plots were very similar in January 2011 and the total P concentration of the soil water from the SRG plot was greater than that of the soil water from the IM plot in February/March 2011.

a)



b)



c)

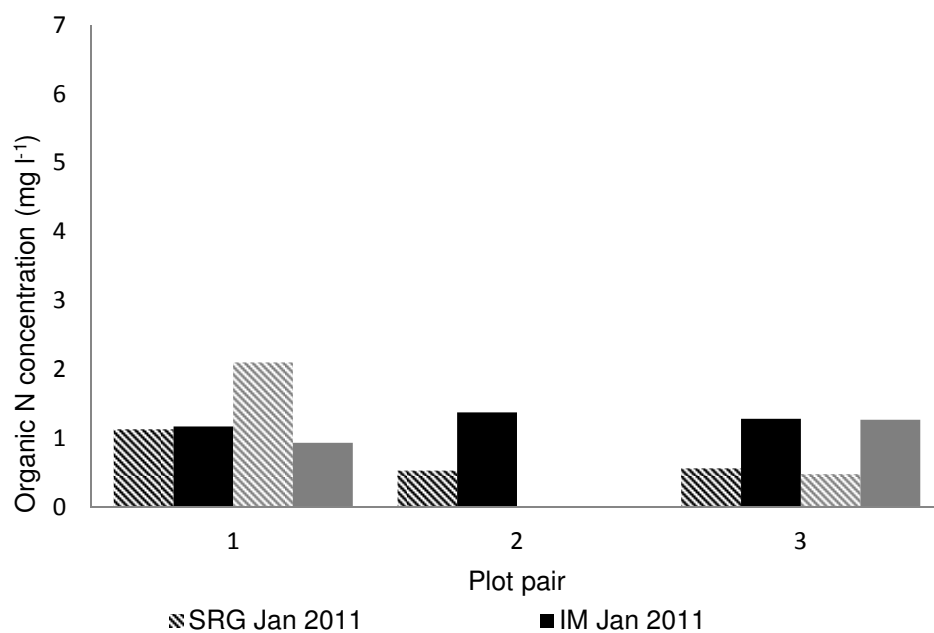


Figure 5.27 Concentrations of a) Total N, b) Total oxidised N, and c) Total organic N, in soil water samples. Soil water samples were collected at NW over a four day period IM and SRG plot pairs 1, 2 and 3 in January 2011 and February/March 2011. An insufficient volume of water for analysis was collected from both the IM and SRG plots of plot pair 2 in February/March 2011.

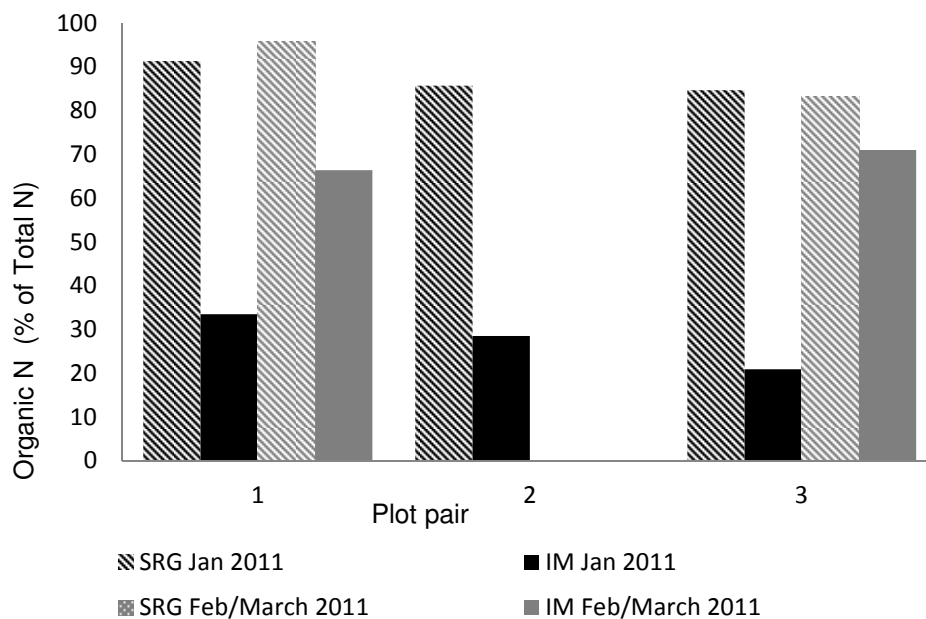


Figure 5.28 Organic N concentration as a % of the total N concentration in soil water samples collected from NW over a four day period in January 2011 and in February/March 2011. Soil water samples were collected from the IM and SRG plots of plot pairs 1 2 and 3. An insufficient volume of water for analysis was collected from both the IM and SRG plots of plot pair 2 in February/March 2011.

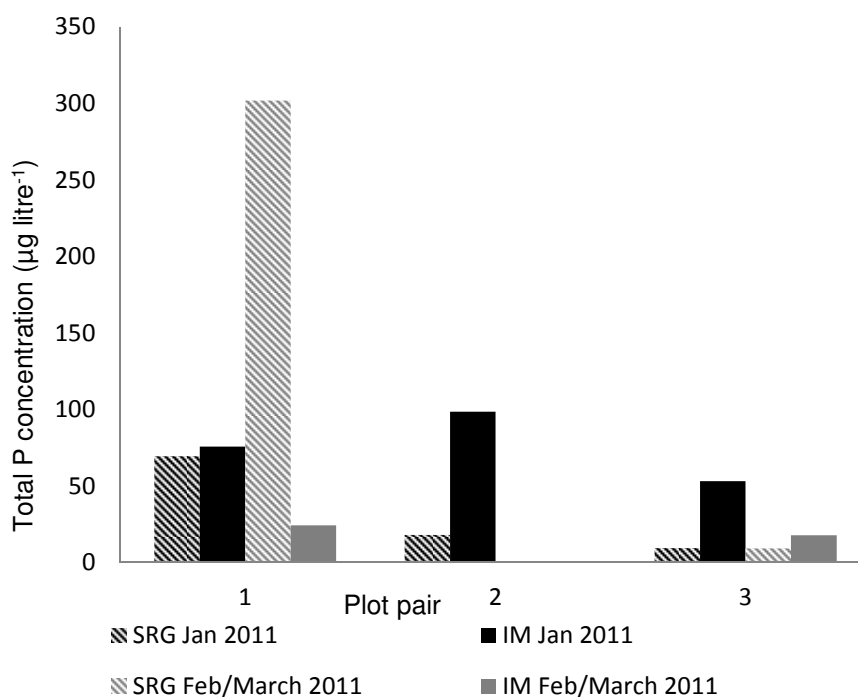


Figure 5.29 Total P concentration of soil water samples collected from NW over a four day period in January 2011 and February/March 2011. Soil water samples were collected from the IM and SRG plots of plot pairs 1, 2 and 3. An insufficient volume of water for analysis was collected from both the IM and SRG plots of plot pair 2 in February/March 2011.

5.8 Discussion

5.8.1 The effect of the cessation of intensive management on plant community diversity and characteristics

The diversity in the NW SRG was much greater than in the paired IM plots, where there was > 99% cover from *Lolium perenne*, and a mean SDI of 0.0157. The species diversity, species richness and % of seed mix species established was very similar across the four NW SRG plots. Many of the same species were identified in all four plots, as would be expected from replicates of the same treatment. Compared to the Scottish plots (Section 4.3), the % cover from sown species tended to be much greater in the NW plots. A possible explanation for this is that the species sown in the SRG at NW were more suited to establishing on former intensive agricultural land. Species that were among the most successful in terms of % cover at the NW plots included *Dactylis glomerata*, and *Phleum pratense*, which in Scotland were only sown in the SRG at site S3. *Lolium perenne*, was another successful seed mix species at NW and was not sown at any of the Scottish sites. These species are typically found growing in managed grassland and around the edges of croplands and all have an EI-N value of 6, indicating they are found on sites of intermediate to high fertility. Their association with higher fertility and lower diversity habitats may explain why they were not included in the Scottish seed mixes, as under the SRDP land managers are required to create species rich grassland and encourage species, other than those typically found to dominate agricultural areas, to establish.

Another difference between the NW and Scottish plots is that the % cover provided by L-forbs was greater in the Scottish plots. This could be the result of high soil P at the Scottish sites (Section 4.3.3), which has been found to increase the dominance of grassland legumes. A great proportion of legumes in the Scottish plots could lead to greater concentrations of potentially mineralisable N being added to the soil. This could limit ES provision to a greater degree at the Scottish plots than at the NW plots.

In 2010 the diversity recorded at the NW SRG plots was greater than at three of the four Scottish plots. The mean % of seed mix species established and species richness

in the four NW plots were also higher than in any of the Scottish plots in 2010. In 2011 the diversity of the NW plots was lower compared to 2010, possibly due to the lower than average rainfall in 2010 leading to increased dominance of drought tolerant species. Over the two years there was no consistent difference in diversity or richness between the NW and Scottish plots.

As with the Scottish sites (Section 4.7.1), the large range of EI-N (2-9) assigned to the species in the NW SRG suggests there may be patchy availability of nutrients, or that species are present in the plots despite sub-optimum conditions. The mean EI-N values for these plots are generally higher than those published by other authors for abandoned intensive agricultural sites. Stevens et al. (2012) studied sites that had been abandoned without fertiliser additions for 15 years and found a mean EI-N of 4.91 for sites with a history of high N addition ($200 \text{ Kg N ha}^{-1} \text{ yr}^{-1}$) and a mean EI-N of 4.49 for sites with a history of no or low N additions (less than $25 \text{ Kg N ha}^{-1} \text{ yr}^{-1}$). The NW SRG only stopped receiving fertiliser additions in 2008, the much younger age of the SRG could be one reason for this difference in mean EI-N of the established species and suggests there is greater level of residual soil fertility in the NW plots. The soil N concentration in the SRG may decrease over time and enable communities more similar to those studied by Stevens et al. (2012) to establish. A mean EI-N of between 4.54 and 4.66 has been found for species-rich UK hay meadows (Critchley et al., 2007) and could be seen as a target for sites such as the SRG plots at NW. The high mean EI-N values found in this study suggest the NW plots are a long way off achieving this target, whilst the findings of Stevens et al. (2012) indicate that, even when abandoned for 15-years, plots with a history of high fertiliser addition may fail to meet this target.

The SDIs for the NW SRG plots are lower than those published for many old, permanent grasslands, which could be seen as target communities. One study in Sweden looked at traditional hay meadows and found SDIs of 2.56-3.71 (Linusson et al., 1998). Other authors have found species diversity to be highly variable across permanent grasslands, with some being less diverse than the NW SRG sites used in this study, for example Janssens et al. (1998) studied old, permanent grasslands in western and central Europe and found them to have SDIs ranging from 0.5 – 5. As

with the Scottish plots, the NW plots have lower plant species diversities than all of the plots described by Linusson et al. (1998) and most of those studied by Janssens et al. (1998). Compared to the diverse grasslands studied by others, the plots in this study also have a higher mean EI-N, indicating the communities are both less diverse and contain species favouring more nutrient-rich environments than those from traditional hay meadows.

5.8.2 The effect of the cessation of intensive management on the composition of the soil microbial community

The data show that the rhizosphere soils of the IM plots in this study had a larger total microbial population, than the SRG soils in. This finding is in contrast to the findings of some authors who have compared PLFA abundance in fertilized and unfertilized grasslands and found the unfertilized plots had a higher abundance of soil microbes (Bardgett et al., 1999; Grayston et al., 2004). A possible explanation for lower soil microbe content in the unfertilized SRG plots in this study compared to the fertilized IM plots is that on establishment of the SRG the soil was ploughed prior to the sowing of a seed mix. Soil cultivation has been linked to reduced soil microbial biomass and the effects of cultivation on soil can be long-lasting (Buckley and Schmidt, 2001), this could explain the reduced soil PLFA concentration in the SRG plots, which were ploughed in 2008. However ploughing often has a greater effect on soil fungal population as it breaks up fungal hyphae networks (Frey et al., 1999). The finding that the bacterial population and not the fungal population was significantly smaller in the SRG plots compared to the IM plots, suggests there is a different factor driving the difference in overall PLFA concentration between the two sites. Another explanation is the change in the plant species composition following sowing of the seed mix in the SRG lead to reduced rates of C substrate delivery to soils (Grayston et al., 2001). Dungait et al. (2013) found that, regardless of the nature of the C substrate, application of C compounds to arable soil resulted in significant increase in the total PLFA concentration. However, whereas Dungait et al. (2013) found that addition of C substrates did not result in increased concentrations of specific biomarker PLFA, this study repeatedly found statistically higher concentrations of bacterial biomarkers in soils from the IM plots compared to the

SRG plots. Possible reasons for the impact of extensification on the bacterial community are discussed more below.

Between 5 March and 11 March 2012 there was a significant increase in the total PLFA concentration in the IM plots. The IM plots received 80 kg N ha⁻¹ nitrate fertiliser on the 9 March, it is likely that this fertiliser stimulated plant growth, leading to increased root exudates, particularly from areas of active root growth at the root apices (Paterson et al., 2006a) which have been shown to lead to transient increases in soil microbial biomass (Lovell et al., 1995), N fertilization has been linked in particular to an increase in root exudate uptake by G-ve bacteria (Paterson et al., 2006b). Application of N fertiliser to N limited soils has also been shown to increase the size of the microbial community due to decreased microbial N limitation (Rinnan and Bååth, 2009), which may partly explain the increase in total soil PLFA concentration following N application in the IM plots.

The second fertiliser application did not lead to a similar increase in total PLFA concentration in the soil of the IM plots, possibly because a smaller quantity of fertiliser was used and there was no peak in soil NO₃⁻ concentration (Section 5.4.2), although other authors have identified a spring maximum in potential in microbial activity (Grayston et al., 2001; Williams et al., 2000), which could have led to a more rapid microbial response to the earlier fertiliser addition. Thus the timing of the soil sampling following the second fertiliser application may not have allowed sufficient time for a response in the microbial community, which may partly explain the lack of a significant effect on the total soil PLFA concentration.

The SRG plots in this study had a higher fungal:bacterial biomarker ratio than the IM plots, due mostly to a lower concentration of bacterial biomarkers in the SRG soils as opposed to a greater concentration of fungal biomarkers. The input of plant derived organic matter to soil has been found to have a significant impact on the composition of the microbial community, which in turn regulates key soil processes such as greenhouse gas fluxes, nutrient cycling and availability, which feeds back to the plant community as well as influencing mineral leaching potential (Paterson et al., 2006b). In particular studies conducted across the UK have found that long-term unimproved grasslands have a greater fungal:bacterial biomarker ratio compared to

semi-improved and improved grasslands (Grayston et al., 2004). However, whereas the smaller fungal:bacterial biomarker ratio in the SRG plots in this study was due to a smaller bacterial biomarker concentration, which was observed along with a smaller total soil PLFA concentration, the greater fungal:bacterial ratios in the unimproved sites described by Grayston et al. (2004) were due to a larger soil fungal biomarker concentration compared to intensively managed sites, which were observed along with greater total PLFA concentrations in the unimproved sites. The greater biomass of fungal hyphae in un-improved / semi-natural grassland soils, leading to greater concentrations fungal PLFA biomarkers, compared to improved grassland soils, has generally been attributed to greater additions of more complex and less labile plant derived carbon compounds from the slower growing plant species typical of nutrient poor soils (Grayston et al., 2004). Experiments using isotopic C labelling coupled with PLFA analysis have shown preferential uptake of more labile, soluble carbon compounds by soil bacteria, whereas insoluble, complex C compounds are metabolised more slowly by the fungal community (Patterson et al., 2008). Plant species data from the NW SRG site in this study showed that the plant species present in the plots were those typically found in more fertile soils and were not indicative of a long term extensively managed, semi-natural grassland, therefore the plant derived organic matter is likely to be relatively high quality and readily mineralized.

The combined findings of the plant and PLFA analyses at the NW sites indicate that the SRG does not resemble a long established semi-natural grassland, despite the higher soil fungal:bacterial biomarker ratio compared to the IM plots. Crucially the fungal population is no greater in the SRG soils, and it is an increasing soil fungal biomass that tends to indicate a shift towards a grassland system with more efficient nutrient cycling and higher N retention. A reason for this lack in a shift in ecosystem characteristics in the SRG plots is that the legacy of intensive agriculture continues to affect soil properties in the SRG plots. A legacy effect was also observed by Bardgett and McAlister (1999), who found that at younger unfertilized grassland sites, where fertiliser had only been stopped for 6 years, there was no difference in the soil fungal:bacterial biomarker ratio compared to intensively managed sites.

The similar ratios of G+ve:G-ve bacteria in the IM and SRG plots in this study is further evidence that the nature of the carbon compounds supplied to both soils is similar (Acosta-Martínez et al., 2008). Typically, unimproved grasslands in the UK have a higher G+ve:G-ve biomarker ratio than improved grasslands. G+ve bacteria tend to show a positive correlation with soil organic matter, whilst soils rich in phosphorus and grasslands dominated by *Lolium perenne* have been found to have a higher concentration of G-ve bacterial biomarkers (Grayston et al., 2004). Generally G+ve bacteria are slower growing and able to utilise more complex carbon compounds compared to G-ve bacteria, thus an increase in G+ve bacteria would indicate a shift towards a more extensive grassland system (Dungait et al., 2011 and 2013). Actinobacteria, a sub-group of G+ve bacteria, have been found to have an important role in the decomposition of organic matter including lignin and can also help improve soil aggregate formation due to their mycelial growth habit (Jenkins et al., 2009). This mycelial growth habit could explain the smaller actinobacteria biomarker concentration in the SRG soils, which had been ploughed four years previously, compared to the soils of the IM plots, which had been under established grassland since the 1980s.

It is possible that the difference in the plant community between the IM and SRG plots in this study is having some effect on the microbial community, as despite the lack of a difference in the fungal biomarker concentration between management types, the IM plots did show a higher concentration of bacterial biomarkers. In this study the existing grassland sward was ploughed up and a new seed mix sown on creation of the SRG. This could explain why the fungal:bacterial biomass was different in the SRG compared to the IM plots in this study and not in the study by Bardgett and McAlister (1999), where the existing sward was kept and fertiliser applications were stopped. In the IM plots in this study 99 % ground cover was provided by *Lolium perenne* a fast growing species typically found in high nutrient environment, whereas in the SRG there was diversity of plant species, some of which were adapted to slightly more nutrient poor environments (Section 5.2). This difference in the plant community could have an important impact on the soil microbes as it has been shown that faster growing species can stimulate the growth of soil bacteria through the secretion of root exudates and the return of high quality

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litter to the soil (Orwin et al., 2010). The reduced dominance by *Lolium perenne* in the SRG plots could lead to reduced supply of plant root exudates which tend to be taken up more by the bacterial community than the fungal community.

The smaller bacterial community in the SRG soils could lead to slower rates of organic matter mineralisation, which, over time, could reduce nutrient availability to plants resulting in a feedback, where by plant species typical of nutrient poor environments, which typically produce lower quality, less labile OM, become dominant, eventually leading to increased fungal biomass and establishment of a more efficient nutrient cycling system with lower rates of loss (Bardgett and McAlister 1999; de Vries et al., 2006) and improved soil structure (Acosta-Martínez et al., 2008). As such the SRG plots in this study could represent an intermediate state with a reduced concentration of soil bacterial but a similar soil fungal concentration to the IM plots, high residual soil fertility may be preventing an increase in soil fungal biomass in the SRG plots as high mineral N availability has been shown to limit fungal growth

5.8.3 The effect of the cessation of intensive management on soil N₂O fluxes

Soil N₂O fluxes throughout the sampling period were low. When peaks in N₂O fluxes were observed these were often only recorded from individual chambers, leading to substantial intra-plot deviation and standard deviations from the mean, such that most large numerical differences between the mean fluxes from the IM field and the SRG proved to be statistically insignificant.

The only occasion on which the mean flux from one site was both larger than 5 g N ha⁻¹ day⁻¹ and differed significantly from the other site was on 13 May 2010, when the mean flux from the SRG plots was greater than from the IM plots. The emissions peak from the IM plots on this date can be explained by the fertiliser application on 23 April, which would have increased available N concentration in the soil and stimulated increased denitrification. However the significantly greater N₂O emissions from the SRG plots, where no fertiliser was applied, are harder to explain. On this occasion, the inter and intra plot variation in N₂O flux is small, indicating that these

emissions peaks are unlikely to be in response to small localised increases in N availability. Samples taken in mid-May 2012 show a slight peak in N₂O emissions from SRG plot 1 but not from either of the other SRG plots, suggesting emissions peaks of the scale of those recorded at the SRG site in mid-May 2010 are not a regular annual occurrence. The unusually low daily maximum temperatures throughout much of 2010 could be one explanation for the peak in N₂O flux from the SRG plots recorded on 13 May 2010. The brief spell of higher temperatures at the end of April 2010 (Section 5.1), could have increased the rates of mineralisation and nitrification in the soil, resulting in an increased NO₃⁻ concentration. The subsequent return to cooler temperatures may have then decreased NO₃⁻ uptake processes among plants and some soil microbe groups and resulted in conditions that gave denitrifying organisms a short term competitive advantage. It is possible that the larger fluxes observed at the SRG site compared to the IM site could result from the different plant communities established at the two sites (Section 5.2). Some of the plant species at the SRG site, particularly those commonly found in less fertile environments (low EI-N), may be less able to increase NO₃⁻ uptake in response to increased availability compared to the rapidly growing *Lolium perenne*, which dominates at the IM sites, resulting in increased availability of NO₃⁻ to denitrifying organisms at the SRG sites. Another possible explanation for the higher N₂O flux from the SRG plots, is a higher soil WFPS compared to the IM plots, as was frequently found from measurements taken in 2012 (Section 5.3). This event highlights the potential for created SRG to act as significant source for N₂O emissions under certain environmental conditions.

On 23 March 2010 and 16 February 2012, the mean N₂O fluxes from both the IM and SRG plots were above 30 g N ha⁻¹ day⁻¹. The fluxes were very similar for all plots at both sites and there was a low intra plot variation, indicating the observed emissions peaks were not due to localised changes in the soil environment. These N₂O flux peaks occurred prior to any fertiliser additions to the IM sites, so the N source for denitrification must have originated from organic or mineral N already present in the soil. One explanation for the fluxes is that they are in response to warming temperatures leading to increased denitrification. This would explain why the peak was observed earlier in 2012 than in 2010, as the February MDMax in 2010 was over 3 °C cooler than in 2012, the MDMax in March 2010 was similar to that in

February 2012. The WFPS at the SRG site on 16 February 2012 was $> 80\%$, this high WFPS is likely to have contributed to the peak in N_2O emissions from denitrification, by increasing the number of anaerobic sites. However the mean WFPS at the IM site was $< 60\%$, rates of denitrification tend to be low when WFPS is below 60%. The N_2O emissions peak from the IM site on this occasion may therefore have originated from a combination of denitrification, nitrifier-denitrification and nitrification coupled denitrification, which have all been found to contribute to N_2O emissions at WFPS of 50- 60% (Bateman and Baggs 2005; Kool et al., 2011).

Following the peaks in N_2O fluxes measured early in 2010 and 2012, very low or negligible fluxes were recorded at the SRG site on subsequent sampling occasions. The WFPS, measured in 2012, was found on most occasions to be over 60%, a figure above which many authors have observed substantial N_2O fluxes (Dobbie et al. 1999; Bateman and Baggs 2005; Russer et al. 2006). The drier weather in 2010, particularly in April-June, may have led to lower WFPS in 2010 compared to 2012, however sampling was concentrated around periods of higher rainfall in both years, consequently it is unlikely that low % WFPS was the main limiting factor preventing substantial N_2O fluxes being observed from the SRG. Low concentrations of NO_3^- and NH_4^+ in the soil were measured throughout 2012. Since the management at the SRG site was the same for all three years (2010-2012) it is likely that available N concentrations were similarly low in 2010-2011 as in 2012. Low NO_3^- and NH_4^+ concentrations are therefore the most likely reason for the very low N_2O fluxes observed from the SRG sites. Findings suggest that any available N being released through mineralisation of organic matter is rapidly being taken up and fixed by the plant or microbial community, or lost through leaching and is not available for denitrification, except for under certain environmental conditions such as those described previously. The occasional larger fluxes from individual chambers at the SRG site, such as occurred in plot 1 on 5 April 2012, are most likely to be due to natural soil heterogeneity, and indicative of areas of higher mineral N content. Localised peaks in available soil N, can also occur from a urine patch from wild fauna. The occasional negative N_2O fluxes recorded from the SRG sites, indicating a net N_2O consumption, are consistent with the findings of other authors who have found that, under conditions of lower available soil N, grassland soils can act as a

sink as well as a source of N_2O (Ryden, 1981; Clayton et al., 1997; Chapuis-Lardy, et al., 2007). Net N_2O consumption may be a response to the presence of anaerobic microsites, leading to N_2O reduction to N_2 , which can occur even under low WFPS within aggregates where oxygen (O_2) consumption exceeds diffusion (Neftel et al., 2007).

Although not statistically different from the mean fluxes observed at the SRG site, there is a tendency for greater mean N_2O fluxes from the IM site on some sampling occasions in 2012. These greater mean fluxes occur following the first application of ammonium nitrate fertiliser of 80 kg N ha^{-1} , when soil nitrate concentrations were greatest at the site, however the emissions peaks were only observed at plots 2 and 3 and fluxes varied greatly between the chambers in each plot. Whilst the N_2O fluxes varied between and within the plots, the elevated soil NO_3^- concentrations were found in all plots. In order to be certain that the NO_3^- and NH_4^+ concentrations of the soil inside the chamber rims were representative of the plot as a whole, additional soil tests were carried out in 2012 and no significant difference was found in N concentration inside and outside the chambers. Thus despite a high NO_3^- concentration throughout the soil following the first fertiliser addition in 2012, the N_2O fluxes were patchy. One explanation for this is that other soil environmental factors, such as the distribution of WFPS and possibly the structure of the microbial community were highly variable at the smaller scale leading to the observed variation in N_2O emissions. Regression analysis identified significant relationships between mean soil temperature and mean N_2O flux for the six plots on some sampling dates, and mean soil WFPS and mean N_2O flux on other dates suggesting that, on some occasions, spatial variation in N_2O flux may have been driven by spatial variation in one of these soil properties. Correlation between variables however does not prove a causal relationship and on all except one occasion the correlation between N_2O flux as the dependent variable and either soil temperature or WFPS as the independent variable was negative. This is contrary to what may be expected, as other authors have found that higher N_2O fluxes are usually associated with higher WFPS (albeit up to a threshold) and higher soil temperature (Smith, et al., 1998b). A larger dataset including measurements taken at smaller spatial scales would help determine potential causes of spatial variation in N_2O fluxes and the

strength and nature of any relationship between N_2O flux and soil temperature or soil WFPS.

Following the second application of ammonium nitrate fertiliser of 39 kg N ha^{-1} , no increase in soil NO_3^- or NH_4^+ concentration was observed and there was no peak in N_2O emissions. There was a slight increase in soil NH_4^+ concentration in mid-May at both the SRG and IM plots, although the increase was much greater at the IM plots. This peak in NH_4^+ concentration coincided with a slight peak in N_2O emissions from plot 1 at the SRG site and all three plots to a varying extent at the IM site. It is possible that the N_2O peaks could be derived directly from the elevated levels of NH_4^+ in the soil, either through nitrifier denitrification by ammonia oxidising bacteria, or through nitrification-coupled denitrification (reduction of the products of nitrification; Kool et al., 2011).

Other management activities undertaken in the IM field, including grazing in 2010 and grazing and fertiliser additions in 2011, did not result in N_2O emissions peaks from the IM plots. WFPS measurements made in 2012 showed that the IM plots tended to have a lower WFPS compared to the SRG plots, and that on several sampling occasions mean WFPS was below 60% at the IM site. The lower WFPS at the IM site could have been a result of a greater water uptake due to higher rates of evapotranspiration from the faster growing, IM sward, compared to the slower sward of the SRG. Where WFPS fell below 60% then the lack of anaerobic pore spaces could have been a limiting factor in denitrification, and hence limited N_2O flux rates.

Even the largest N_2O fluxes observed from the plots throughout the experiment were lower than the peak fluxes recorded by other authors using the static chamber technique in UK grasslands. Conen and Smith (1998) reported fluxes of up to $243 \text{ g N ha}^{-1} \text{ day}^{-1}$ from an IM grassland 10 miles south of Edinburgh. Cardenas et al. (2010) worked on a site adjacent to the SRG plots at NW, and found peak fluxes of around $100 \text{ g N ha}^{-1} \text{ day}^{-1}$ with fertiliser application rates of $200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. Other authors have found fluxes to vary greatly between years, depending on climate; in one wet year Jones et al. (2005) found peak fluxes of up to $340 \text{ g N ha}^{-1} \text{ day}^{-1}$ from a UK grassland receiving three applications of $100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, whilst a drier year at the same site gave peak fluxes of only $19 \text{ g N ha}^{-1} \text{ day}^{-1}$. The high spatial variability

and short duration of the peaks in N_2O flux following fertiliser application to the IM field in this study are consistent with the findings of Neftel et al. (2007). This high variability, both spatial and temporal, in N_2O fluxes indicates that there is a need to carry out further work on a greater number of sites and over a longer sampling period in order to compare the climate regulating service potential of IM grassland and recently created SRG sites. This study has shown that soils under both forms of management have the potential to produce substantial N_2O fluxes. However the unusual weather patterns throughout the sampling period, including the cool temperatures, particularly in 2010, and the low rainfall except for two unusually wet months in April and June 2012, are likely to have affected key soil environmental factors and plant and microbial communities. This could mean the results from this study may differ from those that would have been observed in a more typical year.

5.8.4 Comparing the output from the UK-DNDC model with field measurements

The modelled and measured fluxes over the period were of a broadly similar order of magnitude. However, the weak and slightly negative correlation between the modelled and measured N_2O fluxes on the 18 sampling dates for both sites shows there was little success in using the model to accurately predict the N_2O fluxes. The model predicted a total N_2O flux from the IM plots over the four month period 13 February to 13 June that was over 33 times greater than it predicted for the SRG plots for the same period, whilst the estimated total N_2O fluxes for the same period based on the measured values was only 1.8 times greater for the IM plots than for the SRG. This difference in total flux estimated from the measured values for the two management types, whilst smaller than predicted by the model, is still substantial given that ANOVAs carried out on field measurements showed no statistically significant effect of management type on N_2O flux on any of the individual sampling occasions (Section 5.5.2). The total fluxes estimated from the measured values should be considered with caution however, as they do not take into account the large inter and intra-plot variability in fluxes. The total flux estimations also rely on extrapolation of the curve line to predict fluxes for days on which measurements were not taken. The high temporal variability in N_2O fluxes mean the results of these estimations, whilst indicating a tendency for higher total fluxes from the IM plots,

should not be considered indicative of a significant effect of management type. To mitigate for the dates when field measurements were not taken, measured N₂O fluxes were compared directly with the model output for the same day. The total measured fluxes for the 18 dates were calculated to be over nine times greater than the total modelled fluxes for the SRG plots, whereas the total modelled N₂O fluxes were 1.79 times greater than the total measured fluxes for the IM plots; the model gave a substantial over estimation of emissions from the IM field and a substantial under estimation of emissions from the SRG.

The overestimation of the total measured N₂O fluxes from the IM plots by the model can be largely attributed to the high flux values in the model output for the dates between 10 April and 8 May 2012 inclusive. These peaks in N₂O emissions were not observed in the field measurements taken between these dates. This could in part be due to within field heterogeneity. Nitrous oxide fluxes were found to be highly variable in space over the sampling period. The model output gives a mean daily flux for the entire field and it is possible that, on some occasions, the N₂O fluxes from the sampling points differed substantially from the field average.

The difference between the model output and measured N₂O fluxes could also be due to the unusual rainfall patterns over the period, with a much wetter than average April and a dry May, which came after a much drier and warmer than average start to the year, as described in section 5.1.1. The conditions, which were far from typical, could have caused the plant and soil microbial communities to be under a state of stress and therefore prevented them from responding to subsequent environmental drivers in the normal manner as assumed by the UK-DNDC model. Further empirical data and the studying of stress responses in plants and soil organisms, could help optimize the UK-DNDC model function for extreme climatic conditions. Climate averages for a region could then be input alongside the yearly climate data and where values depart significantly from the long term average, altered “stress response” parameters could be used to model fluxes.

The underestimation of the total measured N₂O flux from the SRG plots by the UK-DNDC model is due both to a measured peak in emissions on 16 February, which was not replicated by the model output and several sampling occasions between 6

April and 8 June, when moderate N_2O fluxes ($0.001 - 0.01 \text{ kg N ha}^{-1}$) were measured in the field, whilst the model output showed no flux.

One possible explanation for this consistent underestimation of background level N_2O fluxes is that the UK-DNDC model is underestimating the soil available N content in the SRG plots, the calculated soil N concentration feeds into the denitrification / nitrification sub-model used to model N_2O emissions (Section 5.6.1). Earlier in the year the measured soil NO_3^- concentrations were greater than the model output values, and later on in the year the measured soil NH_4^+ concentrations were greater than the model output for the SRG sites. Overall the correlation between the measured and corresponding model output values for both forms of available N was very low for both the IM and SRG sites. However the difference between the model output and measured values was greatest for the SRG plots. An explanation for the particularly poor model performance for the SRG site is the specific management history of the SRG site in this study, which prior to 2008 had been under continuous intensive management before being sown with a species rich sward mix and taken out of intensive management for four years. The legacy of the past intensive management, coupled with changes in the plant community could have led to the SRG system functioning very differently, with different rates of nutrient cycling, compared for example to a site in the first year without fertiliser additions which had retained the existing sward. If the sites used to provide empirical data to parameterize the UK-DNDC model for low input management had a contrasting management history to the SRG in this study then this could be the reason for the large differences between the model output and field measurements.

The correlations between the model outputs for soil WFPS and the measured values were relatively strong and significant for both the IM and SRG plots. The model output was closer to the measured values for the IM plots than for the SRG plots, with the measured soil WFPS for the SRG plots tending to be greater than the model output. In order to improve the model output for soil WFPS the UK-DNDC default values for wilting point (WP) and field capacity (FC) were altered from 0.27 and 0.57 respectively to 0.4 and 0.7 respectively following discussions with Li, (2012). At depths below 1 cm, the model output is particularly closely controlled by the FC

input value, the model will not allow soil WFPS to rise more than 3% above the set FC. Changing the values for FC and WP did produce a model output that was closer to the measured soil WFPS for both the IM and SRG plots. The field measurements frequently found soil WFPS in the SRG plots to be greater than 0.7%, suggesting that the FC input value may need to be raised even more relative to the default value, which is calculated based on the known soil clay content. The strong influence of FC and WP on the model output values for soil WFPS, suggests that in the future both these values should be directly measured for a site. The correlation between the measured and modelled values for soil WFPS could also vary depending on the time at which soil samples were collected. The model output gives a single average value for soil WFPS each day, whereas the soil WFPS in the field is likely to vary over the day depending on when soil samples are collected relative to rainfall events and peak temperatures. The model output also gives soil WFPS values for discrete depths, whereas soil samples for moisture content analysis were collected across a depth range of 0-7.5 cm. As such some difference between the model output and measured soil WFPS should be expected.

5.8.5 The effect of the cessation of intensive management on the concentration of N and P compounds in soil water samples

In order to quantify the rates of nutrient loss through leaching it would be necessary to calculate the rate of soil water drainage and run off from field sites and use this alongside the measured concentrations of nutrients in the soil water from that site. A tool such as the IRRIGUIDE (Bailey and Spackman 1996) model could be used to predict the rate of soil water drainage. The limited success at obtaining soil water samples in this study means this technique has not been applied. The differences measured in the concentrations of nutrients in the soil water samples from this study can be used to indicate the potential costs of nutrient loss from the SRG plots relative to the IM plots, assuming they experience similar rates of drainage.

From the limited data collected, the evidence suggests that the concentration of TOxN and total N dissolved in the soil water was greater for the IM plots than the SRG plots, hence there is a potential for greater fluxes of N from the IM plots to surface and ground water. The greater the flux of N in soil drainage and runoff, the

greater the risks of detrimental effects on the amenity value and ecology of surface waters (Søndergaard and Jeppesen, 2007). Nitrogen that enters rivers as oxidised N (TOxN) is readily available for biotic uptake and may have a more immediate impact on aquatic systems close to the N source. Nitrate also poses a specific risk to human health if it enters drinking water supplies (Townsend et al., 2003). Thus if the total N and TOxN concentrations in soil water from SRG sites were found to be consistently lower than in soil water from IM sites, as this evidence suggests, conversion to SRG could have a positive impact on ES' linked to water quality regulation.

The data also provide important evidence about the potential TOrgN and total P fluxes in soil water solution from IM and SRG plots. Both P and less labile N compounds, including many organic N compounds, can accumulate over time in groundwater (Holman et al., 2008) and in sediment deposited in lakes and coastal regions, where they contribute to eutrophication (Seitzinger and Sanders, 1997) and, in the case of TOrgN, can be a source of N₂O emissions (Kroeze and Seitzinger, 1998). Unlike TOxN, the concentration of TOrgN and TP in soil water samples taken from IM plots was not consistently greater than for water samples from SRG plots, in some cases SRG sites may have a risk of greater TOrgN and P fluxes than IM sites. Furthermore TOrgN consistently contributed over 80% of the total N concentration in the soil water from the SRG plots, whereas the % contribution of TOrgN to the total N concentration of soil water from the IM plots was lower. This evidence highlights the importance of measuring both TOxN and total N in soil water samples when assessing the potential costs of nutrient leaching; otherwise there is a risk of substantially underestimating the costs of nutrient losses from SRG sites.

Chapter 6 Discussion

This chapter will review the hypotheses set out in Chapter 1 and assess the extent to which the hypotheses are supported by the results presented in Chapters 4 and 5

6.1 Hypotheses 1 and 2 – the effect of extensification on soil chemical and physical properties

The data from the Scottish field sites, obtained from soil sample analysis, provide the best opportunity to assess the extent of the support for hypotheses 1 and 2. However where available data from NW will be used alongside Scottish data in order to determine the overall extent of the support for the hypotheses.

6.1.1 Hypotheses 1.1 and 2.2 – the effect of extensification on total soil N concentration

All the Scottish sites, with the exception of site 5, were on arable farms, where soils had received mineral fertiliser. The IM field and the SRG at site 5 had been under arable management until 2001, when the entire farm converted to intensive grassland management, four years prior to the creation of the SRG. Hypotheses 1.1 and 2.2 state that:

- 1.1 SRGs created at sites with a history of organic N addition, due to organic fertiliser applications or continuous grassland management will have high residual concentrations of total soil N and C, similar to IM fields, due to long-term nutrient accumulation whilst under the intensive management.
- 2.2 Initially SRGs on former arable land with a history of inorganic fertiliser amendments will have low soil N and C concentrations, due to limited accumulation of organic N during the intensive management. Over the first ten years increased N fixation and higher returns of plant matter to soil in the SRGs will increase total soil N.

Analyses of the total N concentrations in soil samples showed that there were no significant differences in total soil N concentration between any of the paired IM and SRG plots. All the plots had lower total soil N concentrations than have been

recorded by others at long established semi-natural grasslands. According to hypothesis 2.2, it could be expected that of the three Scottish sites that had historically received inorganic fertiliser additions under solely arable management, the older SRGs would have higher concentrations of total N relative to the paired IM plot, however the data do not support this part of the hypothesis. The evidence does appear to support the suggestion that arable management and inorganic fertiliser use can limit soil N accumulation, and result in lower total soil N concentrations, as shown by comparisons with studies of soil N at long established semi-natural grassland in North West Europe. The maintenance of low soil C:N ratios and high available soil N concentrations in the Scottish SRG plots relative to long established SRGs is additional evidence that soil N cycling in the plots is still dominated by mineralisation with low rates of N accumulation even after 10 years of extensive management. Site 5 differed from the other Scottish sites in that prior to the establishment of the SRG the site had been under intensive grassland management for 4 years. According to hypothesis 1.1 both the IM and SRG plot at site 5 could be expected to have higher total soil N and C concentration than was found at the other sites, this was not found to be the case. It is possible that had site 5 been under intensive grassland management for longer prior to sampling then the findings may have been different. Another reason for the lack of any observed difference between site 5 and the other three sites is that site five still received mostly inorganic fertiliser applications and was cut yearly for hay. Thus, the rate of organic matter addition to the soil may not have been greater than at the arable sites. In order to further address hypothesis 1.1 additional sampling at field sites with a known history of high rates of organic fertiliser amendment would be necessary. This would help determine whether organic N input leads to high residual soil total N concentrations in newly established SRGs and if so the subsequent effect on ES service provision.

6.1.2 Hypothesis 1.2 – the effect of extensification on available soil N concentration

Hypothesis 1.2 states that:

Legacy effects of intensive agriculture on the soil C:N ratio, the soil biota, and plant-microbe interactions will maintain high rates of net mineralisation in SRG plots,

similar to those in IM plots, leading to similar concentrations of available N in the soils of IM and SRG plots.

The Scottish data appear to support this hypothesis as no significant difference was found between the soil available N concentrations in the IM and SRG plots in Spring or Summer, whilst the EI-N values for the SRG plots suggest that the plant species establishing are those adapted to more fertile soils. In addition there was no significant difference in the soil C:N ratio between the two types of management.

The data from NW however seem to contradict the hypothesis; repeated soil sampling in 2012 showed two peaks in soil available N concentration, which were significantly larger in the IM plots than in the SRG plots. Additionally soil samples collected throughout spring showed consistently higher soil NO_3^- -N concentrations in the IM plots. The greatest concentration of soil NO_3^- -N ($277 \pm 39.5 \text{ mg N kg}^{-1}$ dry soil) was recorded 14 days after the first nitrate fertiliser application to the NW IM plots, this concentration was 3.6 times more than the greatest concentration of NO_3^- -N recorded at any of the Scottish plots. The second nitrate fertiliser application to the NW IM plots was followed by a peak in soil NH_4^+ -N ($124.8 \pm 7.14 \text{ mg N kg}^{-1}$ of dry soil) measured 29 days later. This was 10.7 times the greatest soil NH_4^+ -N concentration measured at any of the Scottish sites. Corresponding but significantly smaller peaks in available soil N were recorded at the same times in the SRG plots. These data indicate that large, but relatively short-lived, peaks in soil available N occur following fertiliser application to IM plots but also that soils of SRGs can show seasonal increases in available N even in the absence of direct fertiliser application.

Combining the findings from both sites gives partial support for hypothesis 1.2. Unsurprisingly direct application of fertiliser to IM sites does increase N availability substantially for short periods, (a few weeks). Newly created SRGs also experience temporal fluctuations in soil available N but not to the same extent as IM plots receiving pulses of fertiliser. Throughout the year SRG soils maintain soil available N concentrations capable of supporting plant species that require high nutrient availability. In this study, except for the period directly following fertiliser application, soil available N concentrations in SRGs less than 10 years old were not

significantly different to paired IM plots. Low concentrations of total soil N accumulation and a small C:N ratio compared to long established SRGs elsewhere in Europe support the suggestion that available soil N concentrations are maintained at newly created SRGs through high rates of mineralisation.

6.1.3 Hypothesis 1.3 –the effect of extensification on soil P concentration

Hypothesis 1.3 states that:

SRGs will have high soil P concentrations, equal or similar to IM fields, due to accumulation of recalcitrant P forms under intensive management, gradual dissolution of P will maintain similar concentrations of available P in IM and SRG plots.

The data appear to support this hypothesis as no significant difference was found in the soil total or available P concentration between IM and SRG plots. The age of the SRG did not appear to affect the soil P concentration relative to that of the paired IM plot, this finding supports the hypothesis that a period greater than 10 years is required before P concentration shows a significant decline following extensification.

6.1.4 Hypothesis 1.4 –the effect of extensification on soil bulk density

Hypothesis 1.4 states that:

Soil bulk density (BD) under SRG will be similar to that of IM fields due to slow rates of recovery of soil structure following cultivation.

When the data from all the Scottish sites were combined, there was found to be no significant effect of management on soil bulk density. However, unlike all the other measured soil chemical and physical properties, a positive correlation was observed between the difference in soil BD between paired IM and SRG plots and the age of the SRG. The soil BDs in the older SRGs were less than for the paired IM plots, whereas the BD of the soil in the youngest SRG (SRG 3) was greater than in the paired IM plot. Soil sampling conducted at NW found the SRG plots to have a greater soil BD than the IM plots. These findings do not support hypothesis 1.4, as they suggest that the soil BD in SRGs does change significantly in the first ten years following extensification. From the data collected it appears that initially, following

creation of an SRG, the soil BD increases relative to locations remaining under the former intensive management; the soil BD of both the three-year-old SRG in Scotland the 4 year old SRG plots at NW were greater than their paired IM plots. This initial increase in BD could be the result of soil compaction; on conversion to SRG, the plots in this study all had the existing crop residue or grass sward removed prior to the sowing of a seed mix, before the new sward established the soil in the SRG plots would have been bare therefore vulnerable to compaction under heavy rain. Furthermore the use of machinery to spread seed and the cessation of yearly tillage in Scotland could also have encouraged increases in BD relative to the IM fields.

Once the sward has established the data suggest that within five -10 years the soil BD at newly created SRGs decreases and becomes lower than it was under the former IM management. This decreasing soil BD could be attributed to increased soil break up by plant roots and macro-fauna, decreased machinery movement and increased addition of soil organic matter to the soil whilst under extensive grassland management. Over time soil BD could continue to decrease in SRGs, but from the data it is not possible to predict how long such changes could continue before the legacy of intensive management on soil BD is no longer apparent.

Intensive management does affect soil BD and from the data it appears soil BD takes several years to decrease following conversion to SRG, however the rate of change is faster than predicted by hypothesis 1.4, as within 10 years the SRG plots in this study did show a significantly lower soil BD than sites under continuous intensive management.

6.1.5 Hypothesis 2.1 –the effect of extensification on soil organic matter concentration

Hypothesis 2.1 states that:

Soil organic matter (OM) concentration will increase at SRG sites following conversion from IM, due to an increased return of organic matter to soil, which accumulates over time.

Analysis of the data showed no statistical difference in soil organic matter concentration between the IM and SRG plots, although there was a tendency for a slightly greater soil organic matter concentration in the top 10 cm of the SRG plots compared to the IM plots. When considered alongside the available soil N and C:N ratio data, the most likely explanation for the limited organic matter accumulation in the SRG plots is that most of the organic matter added to the SRG soil is being rapidly mineralised. The plant species identified at the SRG plots are rapidly growing species, typical of fertile soils, which produce litter of high quality, with a low C:N ratio that is rapidly cycled. The hypothesis that organic matter mineralisation is relatively rapid in the newly created SRGs is supported by the relatively high available soil N concentrations compared to long-established SRGs. This relationship between organic matter quality and nutrient supply is likely to be sustained for many years following SRG creation by positive feedback as high soil available N concentrations maintain the rapidly growing plant species characterised by high quality litter which is rapidly mineralized (Hobbie, 1992). Thus the bulk soil C:N ratio is very slow to increase and organic matter accumulation is very slow; this could explain why there is only a slight increase in soil organic matter concentration in the SRG plots relative to the IM plots in this study.

6.1.6 Hypothesis 2.3 – the effect of extensification on soil pH

Hypothesis 2.3 states that:

Increased organic matter to SRG soils will increase the supply of organic acids to the soil leading to a decline in soil pH. Additionally the cessation of lime applications, which are applied when necessary under intensive arable management, will lead to a gradual decrease in soil pH particularly in the rooting zone (top 10 cm) of SRG plots compared to plots remaining under intensive management.

The data partially support this hypothesis as the soil pH in the top 10 cm of the SRG plots tended to be slightly lower than in the paired IM plots in both the spring and the summer, however the soil pH in the 30-40 cm was greater in the SRG plots than the IM plots, statistical analysis identified a significant interaction effect of management and soil sample depth on soil pH. It is likely that this interaction is due to the cessation of tillage in the SRGs, which halts the mixing of the upper and lower soil

leading to stratification. It is not possible to determine whether the increased organic matter additions and the cessation of liming also contribute to the slightly lower soil pH in the SRG surface soil as hypothesised. However, the difference in surface (0-10 cm) soil pH between the paired IM and SRG plots is small (<0.2 units) and not universally observed, the difference between the IM and SRG plots is greatest in the 30-40 cm soil depth range. Thus reduced mixing of soil layers, rather than increased acidification in the rhizosphere, appears to be the main cause of the differences in the soil pH profile between management types. Over longer time periods (more than 10 years) however SRG soils could become increasingly acidic, if this is the case then changes to soil pH could impact soil nutrient cycling and ES provision at older SRGs.

6.2 Hypothesis 3 – the enhancement of biodiversity provision following SRG creation

Plant species data from both the Scottish and NW plots can be used to assess the extent of the support for Hypothesis 3, which states that:

High soil nutrients and lack of a local seed bank will limit the establishment of desired plant species with few species from sown seed mixes establishing. Land cover at SRG sites will be dominated by invasive grasses and species indicative of fertile soils.

The data from the Scottish sites broadly support the hypothesis as non-sown grass species provided at least 38% of the plant cover in all SRGs on each sampling date and on some occasions they provided more than 60% of the plant cover. The total cover from sown species never exceeded 50%. Of the species sown in the SRG seed mixes the % that established varied between sites and from year to year from 23.1 – 61.5 %. Analysis of the EI-N values of the identified plant species generally supports the hypothesis that established plant species would be those favouring high fertility soils, although the wide range of EI-N values indicates that some species characteristic of less fertile soils did establish in the newly created SRGs.

In the NW SRG plots a greater amount of cover was provided by sown species, which consistently provided more than 75% of the land cover. Of the species present

in the seed mix over 60% were identified in each SRG plot. As with the Scottish SRG plots the mean EI-N values of the established species were greater than those of long established SRGs although some species with low EI-Ns were present in the plots. It is likely that the greater dominance of sown species in the NW SRGs compared to the Scottish SRGs is due to the type of species sown; of the grass species sown at NW the most successful were those typically found in agricultural settings on highly fertile soils. The species sown in the Scottish plots tended to be of higher conservation value but were not as successful at establishing.

The plant species diversity in all the SRGs in this study was lower than in long established SRGs elsewhere in Europe. The SDI varied between years, and comparisons between sites suggest that grazing and the presence of bare ground can encourage greater diversity. There was no observed correlation between soil fertility and species richness; it appears that high nutrient concentrations in the soil affect the nature of the plant community leading to dominance by rapidly growing grasses with high EI-N, whilst diversity is more affected by management and factors such as drought.

6.3 Hypothesis 4 – the effect of extensification on the size and composition of the soil microbial community

Hypothesis 4 states that:

The size and composition of the microbial community at SRG sites younger than 10 years old will be similar to IM sites due to the slow rate of changes in the soil microbial community following conversion to SRG.

The data from the PLFA analysis carried out on soil samples from the NW plots partly refute this hypothesis as the size of the microbial community was found to be significantly greater in the IM plots on three out of four sampling dates, whilst the soil fungal:bacterial biomarker ratio was found to be greater in the SRG plots on three of the four sampling occasions. However, crucially, the fungal biomarker concentration was no different between the two management types and it is a larger soil fungal biomass that has been linked to high nutrient use efficiency, reduced N and P losses and improved soil structure in long established extensive grasslands

(Bardgett and McAlister 1999). Additionally, within the bacterial community the relative proportions of G+ve:G-ve species were not found to be affected by the management, further indicating that the chemistry of carbon compounds in the soil are similar in the IM and SRG plots.

The main contributor to the difference in total PLFA concentration and fungal:bacterial biomarker ratio between the IM and SRG plots in this study is the greater concentration of bacterial PLFA biomarkers in the soils of the IM plots. It is possible that this could be partly due to the cultivation that occurred at the SRG plots prior to sowing the seed mix, which may have reduced the bacterial biomass; however this would not explain the lack of effect on the fungal biomarker concentration. Another explanation is that the change in the plant species composition on establishment of the SRG, and possibly a slower plant growth rate due to absence of fertiliser application, led to a reduction in the plant root exudates and a reduced stimulation of bacterial growth within the rhizosphere. Thus whilst there is some difference in the soil microbial community in the IM and SRG in this study some key indicators of a change in ecosystem function and nutrient cycling efficiency remain unchanged.

6.4 Hypothesis 5 - the effect of extensification on field measurements of N₂O and the output from the UK-DNDC model

Hypothesis 5 states that:

No or limited decrease in soil N content and little change to the soil microbe community will lead to similar background N₂O fluxes from both IM and SRG sites when determined through in-field measurements and modelling using the UK-DNDC model. Short lived N₂O emission peaks, associated with direct fertiliser application, will be observed from the IM plots.

The data partially support the hypothesis in that there was rarely any significant difference in N₂O flux between paired IM and SRG plots in Scotland or at NW. On most measurement dates the measured fluxes were very low or negligible, thus the occasional emissions peak, even when short lived will provide a substantial

contribution to the total yearly flux from any one site. As hypothesised there were substantial peaks in emissions at times from the IM plots; however these were highly spatially variable, and application of fertiliser N to IM plots did not always lead to peaks in emissions. In addition to the hypothesised N₂O emissions peaks from the IM plots, there were at times substantial peaks in N₂O emissions from SRG plots. Despite them receiving no fertiliser applications, there were also emissions peaks recorded at the IM plots at NW prior to the first fertiliser application of the year. The observation that substantial N₂O emissions peaks can occur in the absence of fertiliser application is an important one as it highlights that the soils of newly created SRGs can still be a significant source of N₂O. Compared to the SRG plots in this study the IM plots showed a slight tendency for greater total yearly N₂O emissions, however the high spatial and temporal variability in N₂O fluxes meant that data analysis found the difference was rarely significant. Even following fertiliser N application the N₂O fluxes did not show a significant peak from all chambers in all plots, suggesting that under some soil and climate conditions fertiliser applications can be made that do not lead to substantial N₂O emissions. It should be noted that the most intensive sampling in this study was carried out in the NW plots in 2012, the unusual climate in the area during this year means that the findings may not be applicable to years in which the climate was less extreme.

With regards to the model predictions, in contrast to the field measurements and in refutation of the hypothesis, the UK-DNDC predicted substantial differences in N₂O fluxes from the NW IM and SRG plots. The correlations between the model predictions and measured fluxes were weak. This may have partly been due to the extreme climatic conditions in 2012 preventing the grassland soil from functioning 'normally' according to the model parameters. Another problem with the model was that it was unable to predict a soil WFPS that was more than 3% above the FC, which could have had a knock on effect on the N₂O predictions and how they compare to measured fluxes at times when WFPS was well above the FC.

In particular the model greatly under-predicted the N₂O fluxes from the SRG plots. One explanation for this is the lack of consideration of land use legacy within the

UK-DNDC model, which could have continued to effect N₂O fluxes from the SRG plots.

6.5 Hypothesis 6 – the effect of extensification on dissolved N and P concentrations in soil water

Hypothesis 6 states that:

High residual soil P and sustained N availability will result in similarly high concentrations of dissolved N and P in soil water samples from SRG and IM sites

The minimal success of soil water sample collection means there are limited data with which to test this hypothesis. The soil water samples that were collected from the NW plots appear to refute the hypothesis as total N and P concentrations in the soil water tended to be greater in the IM plots. However one important observation from the data is that over 80% of the total dissolved N in the soil water collected from the SRG plots was organic N. This emphasises the need for future work on N loss to include analysis for organic N as well as inorganic N.

Insufficient sample volume was collected for analysis of total dissolved N and P concentration in soil water from the Scottish sites, therefore the effect of management shift from intensive arable to extensive grassland on N and P leaching cannot be directly determined. Soil chemical analysis showed similar concentrations of NO₃⁻-N, total N, available P and total P in the soils of paired IM and SRG plots which could suggest for much of the year the risks of N and P leaching under both types of management are similar. However it should be noted that immediately following fertiliser application to IM plots there could be temporary peak in soil mineral N concentration, as observed in the NW IM plots, which could lead to a short term increased risk of leaching. A full comparison of the potential nutrient leaching risk from IM and SRG sites would need to include peaks in mineral leaching associated with fertiliser application.

Chapter 7 Conclusions and further work

The aim of this research was to assess the potential for enhanced ES provision following creation of SRGs on former intensively managed agricultural land. The focus was on SRGs that were less than 10 years old as in the UK farmers enter into agri-environment schemes for a period of between five and 10 years. Therefore it is important to assess whether the potential benefits in terms of enhanced ES provision from newly created SRGs during this time period are sufficient to justify 1) the financial subsidy received through the CAP 2) the reduction in productivity particularly in the UK where a high population density puts pressure on limited land supply.

The objective was to provide preliminary data from a small scale study that could be used as an initial assessment of the potential benefit of extensification schemes and to inform further work.

The comparison of the soil chemical and physical properties of paired IM and SRG sites has provided evidence of a legacy of intensive management which lasts for at least 10 years following extensification. There was no significant difference in soil nutrient contents between the paired IM and SRG sites in Scotland and, whilst analysis in Devon showed that peaks in soil available N occur in response to fertiliser application at IM plots, these peaks were short lived and plant species surveys indicated soil fertility in the SRG plots remained high and as such was affecting plant community composition at the sites.

The existence of a legacy effect of intensive management on soil properties identified by this research has highlighted the need for further work. Sampling from a wider variety of SRG sites with histories of both intensive arable and grassland management over a longer period of time would help determine the duration of this legacy and whether its size and duration is affected by factors such as soil type or the nature of the former management.

The PLFA analysis carried out in this study gave an insight into broad scale changes in the microbial community that occur following extensification schemes.

Methodological difficulties including contaminated standards meant that it was only possible to conduct PLFA analysis on soil samples from the NW plots. Repeating the protocol for the Scottish plots would provide an interesting comparison between the two types of land use change, namely intensive arable to extensive grassland (Scottish sites) and intensive grassland to extensive grassland (NW sites). The PLFA method for assessing changes in the microbial community is often favoured as it is relatively quick and inexpensive compared to other microbial analysis techniques and also selects only for live microbial cells due to the rapid breakdown of phospholipids following cell death (Frostegård et al., 2011). An extension of the PLFA technique using isotopic labelling of C substrates could provide further information on which parts the microbial community are most metabolically active under different types of management (Dungait et al., 2011). There are however limitations to the PLFA method, for example the level of specificity of certain biomarkers has been drawn into question. Also where a change in concentration of a certain PLFA is observed it could be due either to a change in the fatty acid composition of a membrane within a species or represent a true change in the species present in the soil. Finally PLFA analysis cannot be used to determine microbial diversity or show changes in the abundance of lower level taxonomic groups as a single PLFA can be common to many species (Frostegård et al., 2011; Zelles, 1999).

A greater understanding of the effect of extensification on the soil microbial community and its function could be gained by recent and on-going improvements in genetic sequencing and extraction techniques. In the future techniques such as analysis of the messenger ribonucleic acid (mRNA) content of the soil could be used to assess the impact of management change on the soil community. This method has greater phylogenetic resolution compared to PLFA analysis, and comparison of mRNA sequences to known data bases of functional genes and taxon specific tags can directly link the microbial community composition to ecological function (Cardenas and Tiedje, 2008; McGrath et al., 2008;).

The finding that background N₂O fluxes were not significantly different from the soils of paired IM and SRG plots suggests that, in the absence of N₂O emissions peaks directly associated with fertiliser applications, extensification will lead to

limited reductions in total N₂O emissions. This study has highlighted the substantial temporal and spatial variability in N₂O fluxes from sites under both IM and SRG management. This will inform future work as it emphasises the need for regular sampling from a large number of chambers. An extension to the work carried out for this study would be to compare net CO₂ and CH₄ fluxes from IM and SRG plots as both are also important GHGs.

There was limited success in obtaining soil water samples using the micro-rhizon samplers during this study. Sufficient water for analysis was only obtained during periods of high rainfall and lower temperatures in Devon where the soils were relatively clay rich. This was despite repeated attempts to collect samples during rainfall events in Scotland. The samplers are also very vulnerable to damage by livestock and must be used in conjunction with a soil water flux model such as irriguide in order to convert nutrient concentrations in soil water solution to rates of nutrient leaching loss. These restrictions on the usefulness of the soil water samplers used signify that an alternative method of calculating nutrient fluxes in leachate and run-off would be preferable. One such method could involve the hydrological isolation of an experimental plot in order to collect runoff and leachate into drainage ditches, through which flow rate and the concentration of dissolved and particulate N and P containing compounds could be measured, such is the set up at the recently established North Wyke Farm Platform (Eludoyin et al., 2012).

In conclusion the findings from this study suggest that the benefits of short term extensification schemes could be limited by the legacy of intensive agriculture on soil properties and nutrient flux processes. As such any enhancement in ES service provision may not be sufficient to mitigate the loss in productivity. Continued advancements in farming practice (McDowell et al., 2001b) and the introduction of nitrification inhibitors such as DCD, which have proven effective at reducing soil N₂O emissions and nitrate losses from fertilized sites (Weiske et al., 2001), will continue to reduce the negative impacts of intensive agriculture allowing production to be maintained with fewer negative impacts. A review of the literature has shown that it can take several decades for soil properties and function to be restored following intensive agriculture, as such more financial and land resources should be

focused on longer term extensification schemes and protecting the remaining areas of truly species rich, infertile grasslands that exist in Europe, as this preliminary evidence highlights how these mature ecosystems cannot be substituted for by newly create SRGs.

There is a need for further long term studies to obtain more data which can be used to assess the overall benefit of grassland creation and restoration under agri-environment schemes. Ideally if time and resources allowed these studies would measure the provision of a wide range of ES' from multiple sites, and combine both long term monitoring at individual sites to incorporate the period prior to and post extensification, along with additional studies from paired IM and SRG sites, as utilised in this study forming a longer chrono-sequence of SRGs in order to determine the length of time needed to fully restore ecosystem function following cessation of intensive agriculture. Investigations at a wider range of sites would also identify the effect that the precise history and nature of the intensive management at a site has on the duration and magnitude of legacy effects on ES provision. In addition sampling at sites with a history of permanent extensive grassland management would help identify a potential 'end-point' or target for the provision of ES', including nutrient cycling efficiency, water quality regulation and reductions in GHG emissions from SRG sites, this would help determine the duration of legacy effects of intensive agriculture.

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Appendix A: Results from soil texture analyses at Scottish sites

Soil samples collected from the Scottish sites on the initial site visit were analysed using a particle size analyser, as described in section 3.6.2, to determine the soil texture. The analyses were carried out in order to verify that the soil texture was similar at paired IM and SRG plots. The results from the analyses are presented here.

Appendices

Site S3

Table A1 Soil particle size distribution at for the IM and SRG plot at site S3.

IM		SRG	
Particle diameter (μm)	Cumulative frequency	Particle diameter (μm)	Cumulative frequency
1	2.67	1	2.24
2	6.57	2	5.52
4	12.4	4	10.6
8	20.8	8	18.8
16	31	16	29.5
32	40	32	39.6
63	50.7	63	52.5
125	64.6	125	69.2
250	83.8	250	87.7
500	96.6	500	98.7
1000	99.3	1000	100

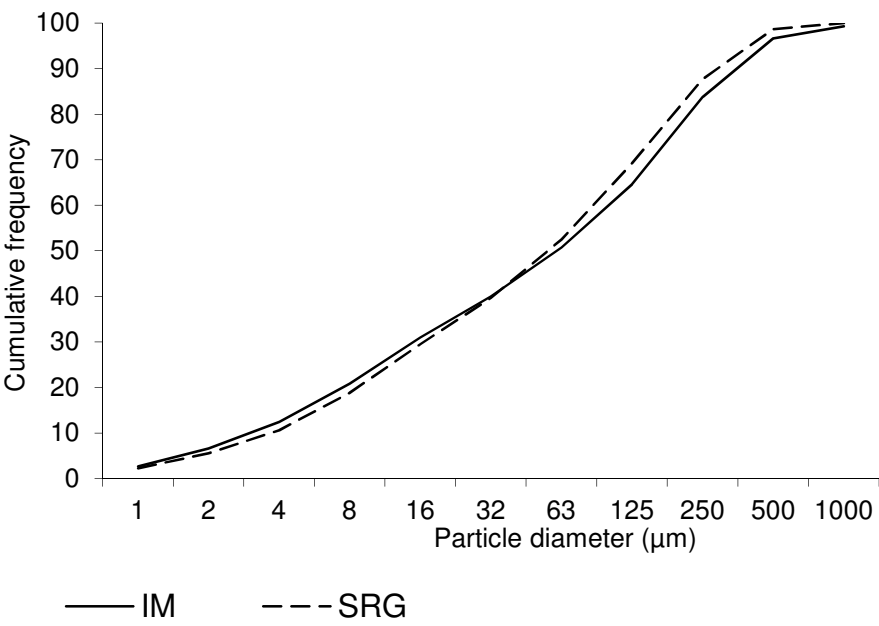


Figure A1 Cumulative frequency graph for soil particle diameter for the IM and SRG plot at site S3.

Appendices

Site S5

Table A2 Soil particle size distribution for the IM and SRG plot at site S5.

IM		SRG	
Particle diameter (μm)	Cumulative frequency	Particle diameter (μm)	Cumulative frequency
1	3.5	1	4.12
2	8.55	2	9.71
4	16.7	4	17.5
8	30.4	8	28.6
16	48.6	16	42.5
32	64.2	32	55.6
63	76.4	63	67.7
125	86.1	125	78.6
250	94.5	250	88.6
500	98.9	500	95.2
1000	100	1000	98.2

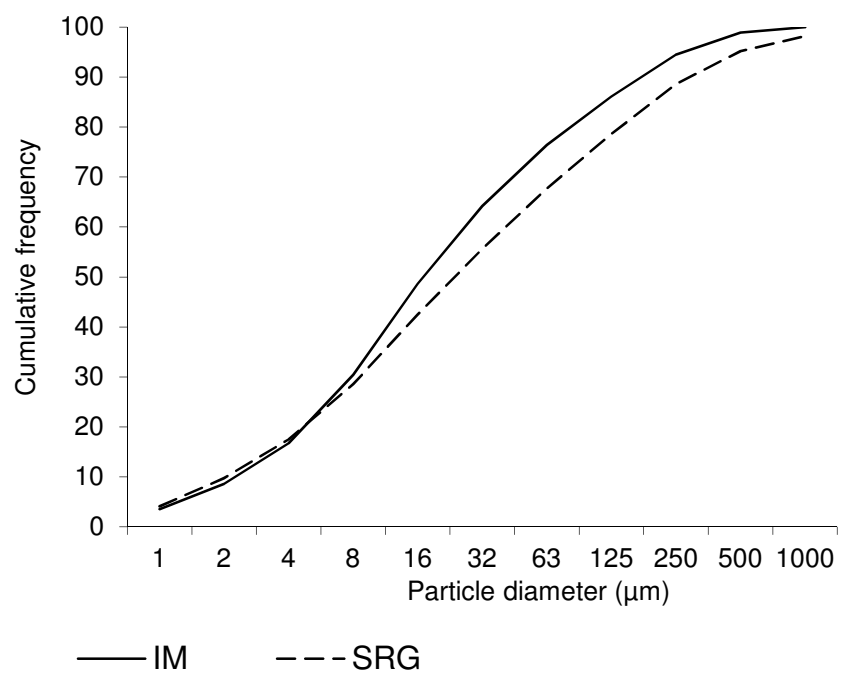


Figure A2 Cumulative frequency graph for soil particle diameter for the IM and SRG plot at site S5.

Appendices

Site S8

Table A3 Soil particle size distribution for the IM and SRG plot at site S8.

IM		SRG	
Particle diameter (μm)	Cumulative frequency	Particle diameter (μm)	Cumulative frequency
1	3.74	1	3.11
2	8.89	2	7.44
4	16.1	4	13.7
8	26	8	23.3
16	37.5	16	36.1
32	47.9	32	48.6
63	58.8	63	61.3
125	71.8	125	76.1
250	86.6	250	91.6
500	95.6	500	98.9
1000	98.6	1000	100

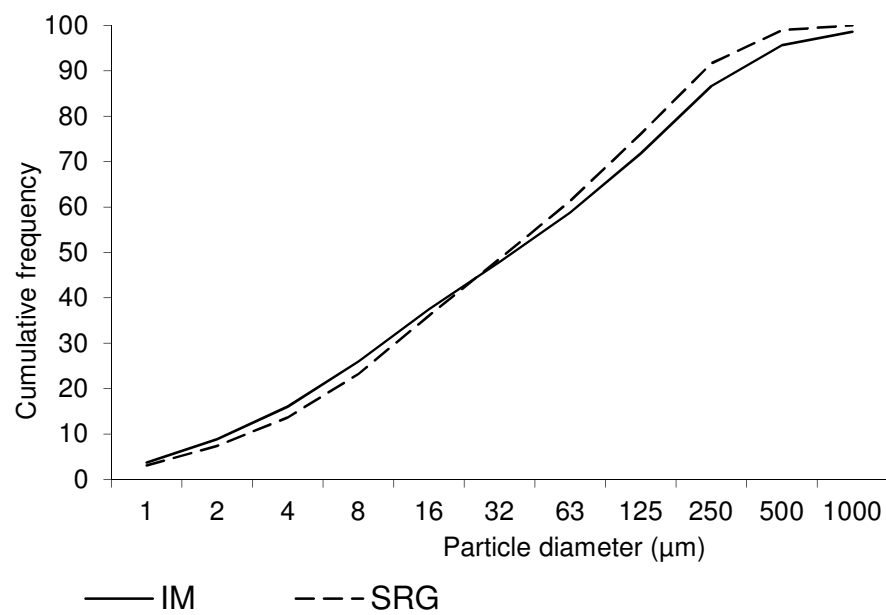


Figure A3 Cumulative frequency graph for soil particle diameter for the IM and SRG plot at site S8.

Table A4: Soil particle size distribution for the IM and SRG plot at site S9.

IM		SRG	
Particle diameter (μm)	Cumulative frequency	Particle diameter (μm)	Cumulative frequency
1	2.68	1	2.81
2	6.51	2	6.71
4	12.5	4	12.6
8	22.2	8	21.8
16	35.3	16	33.9
32	48.5	32	46.2
63	62.1	63	60.3
125	75.4	125	76.3
250	88.4	250	91.7
500	95.4	500	98.4
1000	98.4	1000	100

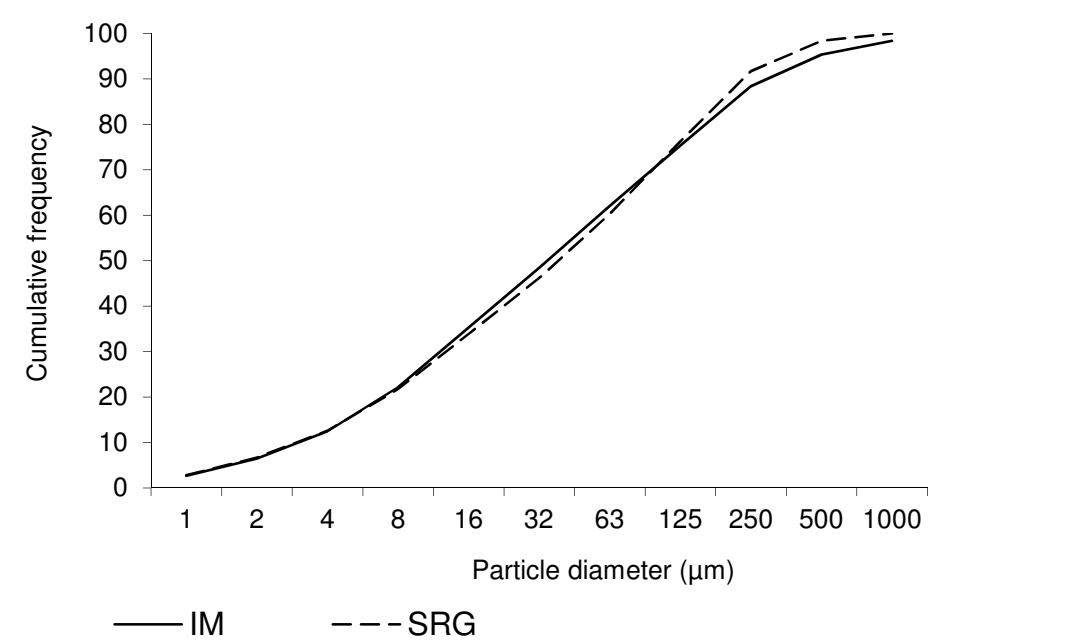


Figure A4 Cumulative frequency graph for soil particle diameter for the IM and SRG plot at site S9

Appendix B: Detailed method for soil water sample collection

Two Prenart ‘super quartz’ soil water samplers (Prenart Equipment Aps, Frederiksberg Denmark) were installed in March-April 2010 in each plot. These samplers were chosen as the PTFE material does not retain minerals such as phosphorus. In addition the small size of the samplers minimised disturbance on insertion and made them more suitable for stony soils. Samplers were inserted at a depth of 0.4-0.5 m. This was the depth to which it was possible to consistently core to at all sites to enable sampler insertion, and was towards the base of the leaching zones to enable comparisons of leachate fluxes between sites. Samplers were installed according to the manufacturer’s instructions. Prior to inserting in the field, the samplers were conditioned by placing under a vacuum in a thin slurry of silica flour and de-ionised water. Gloves were worn when handling the samplers at all time to prevent contamination. Soil water samplers were located close to two of the gas sampling chambers to assist in locating the samplers again once buried (Figure 3.5.1). Pits were dug to 0.35 m by hand then a 20 mm soil corer was used to core to a total depth of 0.5 m below the soil surface. The sampler tube was inserted into a protective rubber outer tubing and string was tied to its top to relocate it. The sampler was inserted carefully into the cored hole and a thick slurry, made from the sieved soil from the pit and de-ionised water, was poured around it to try to ensure a good seal. The soil pit was filled in, taking care to leave the end of the sampler tube and the string at the surface and protect them within a partially buried up turned pot. The location of the samplers was marked with a cane. The samplers in the IM plots were pulled up and re-deployed as necessary prior to ploughing.

Collecting and analysing water from Scottish sites

The samplers were left for at least three weeks before any attempt was made to obtain a sample to allow time for ionic concentrations in soil water around the samplers to equilibrate with the rest of the soil. Soil water collection was carried out during periods of heaviest rain. Samples were collected in 1 l polypropylene bottles (Prenart, Frederiksberg Denmark) to which a vacuum of – 100 kPa was applied using a portable vacuum pump. The collecting bottles were sealed and left attached to the sampler in the field for up to 96 hours to try and obtain sufficient water for analysis.

On only one occasion was sufficient water for analysis obtained from a Scottish site. On this occasion the water samples were stored at 4 °C for 2 days according to York and McHale (2000) and analysed for NO_3^- -N, NH_4^+ -N and PO_4 -P concentrations at The University of Edinburgh using a Bran & Luebbe Auto Analyser III and the same methods described in sections 3.6.6 and 3.6.7. There was insufficient sample to analyse for total N concentration in the sample and therefore derive organic N.

Installing soil water samplers in North Wyke plots

Two Prenart soil water samplers were installed in each IM plot near to the gas sampling chambers at 0.5 m depth as described earlier although a motorised corer was used to core from the surface to 0.5 m. The sampler tubing and string were buried in a trench 40 mm deep with the ends protected inside a partially buried upturned plastic pot with a hole in the lid enabling easy access for sample collection. Samplers had already been installed using this same method in the SRG plots as part of the on-going wider experiment at the SRG plots.

Collecting and analysing water from soil water samplers at North Wyke

On each sample occasion a vacuum of -1 kPa was applied to the collection bottles, which were then sealed and left at the soil surface attached to the samplers for up to 96 hours to allow for a sufficient volume of sample to be collected. The timing of and protocol for collection from the IM plots was identical to that used at the SRG plots where soil water samples were already being collected and analysed for inorganic N and total P content (additional analysis for total N was carried out on the samples from the SRG plots being used in this investigation). All samples were refrigerated and analysed by lab staff at North Wyke within 1 week of collection for total oxidised N (NO_3^- -N + NO_2^- -N), NH_4^+ -N, total P, and total N. Total organic N was derived by subtracting total oxidised N and NH_4^+ -N from total N.

Appendix C: Map showing layout of existing extensification experiment at North Wyke, with location of sample plots

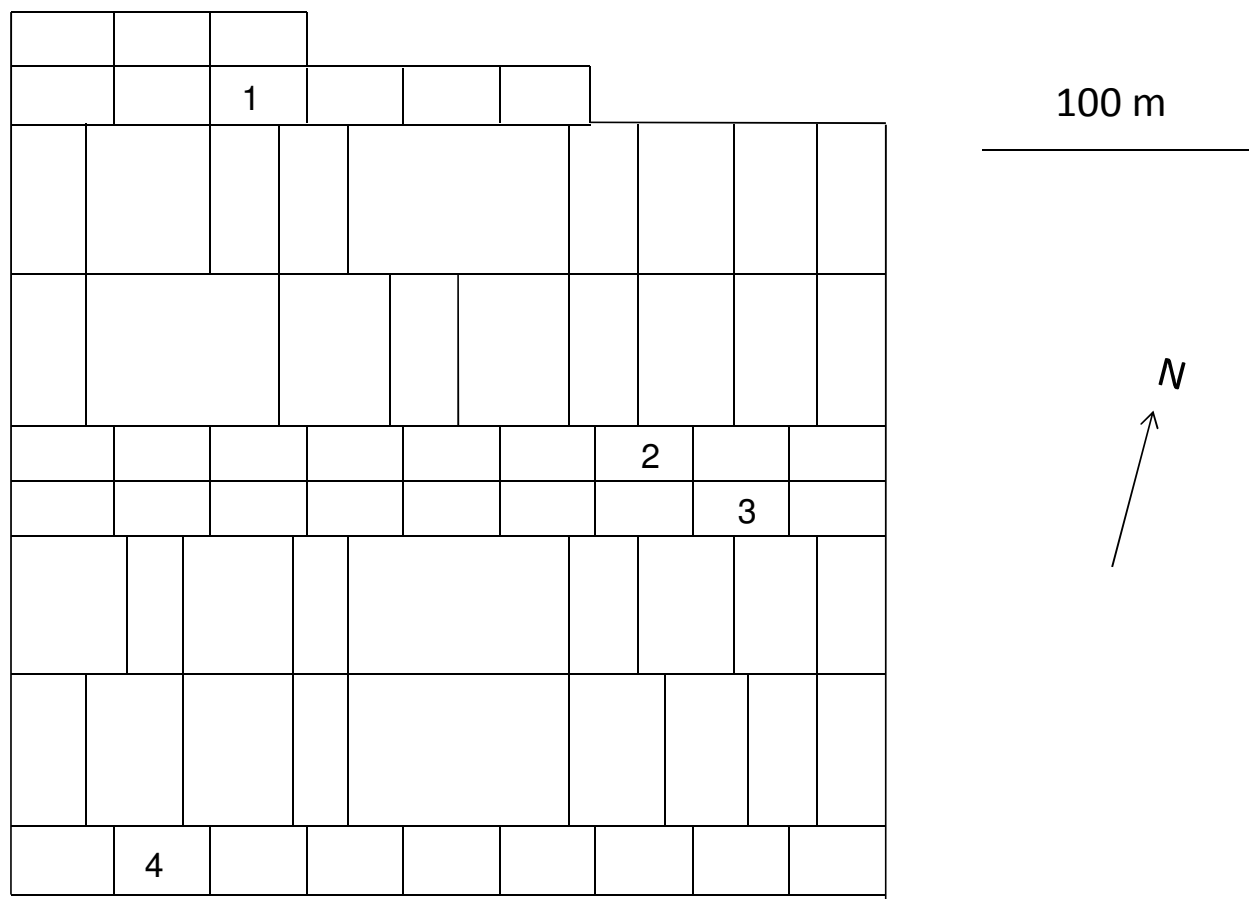
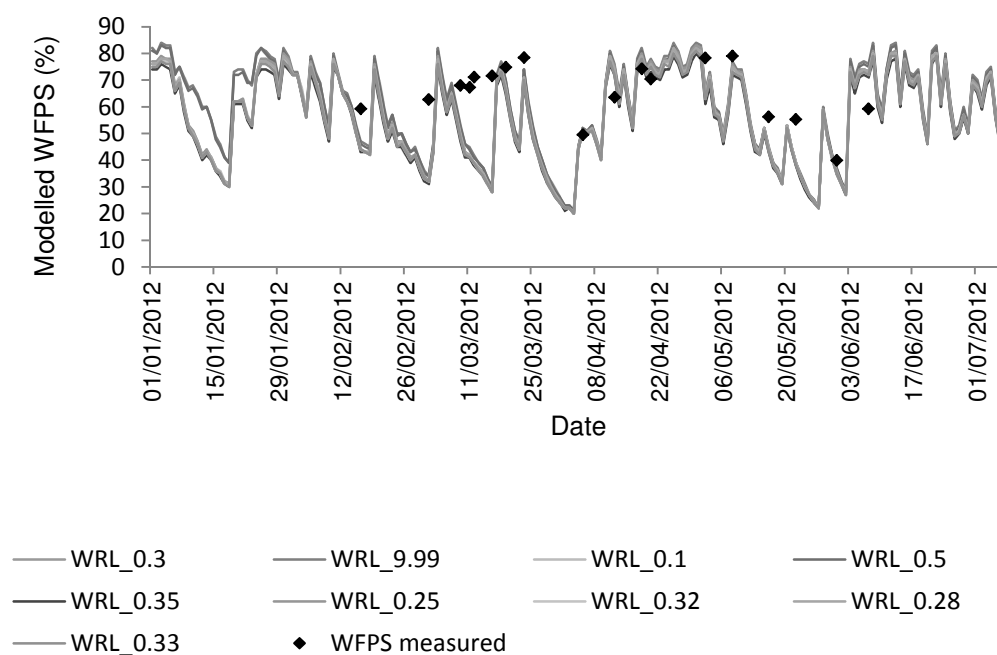


Fig C1 Map of existing experimental site at North Wyke and location of SRG plots 1-4.

Appendix D: Optimising the input value for WRL depth in the UK-DNDC model

a)



b)

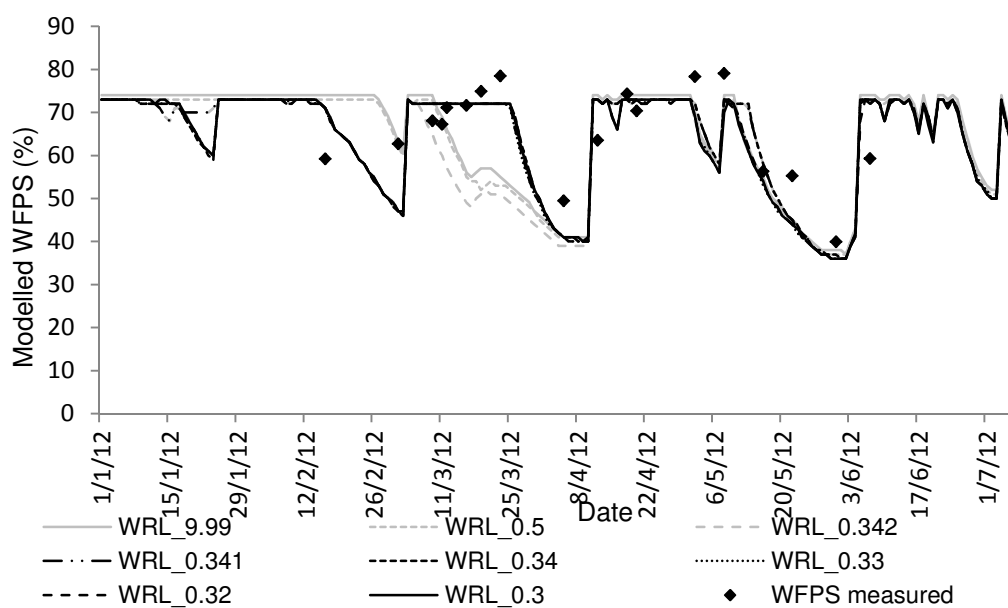
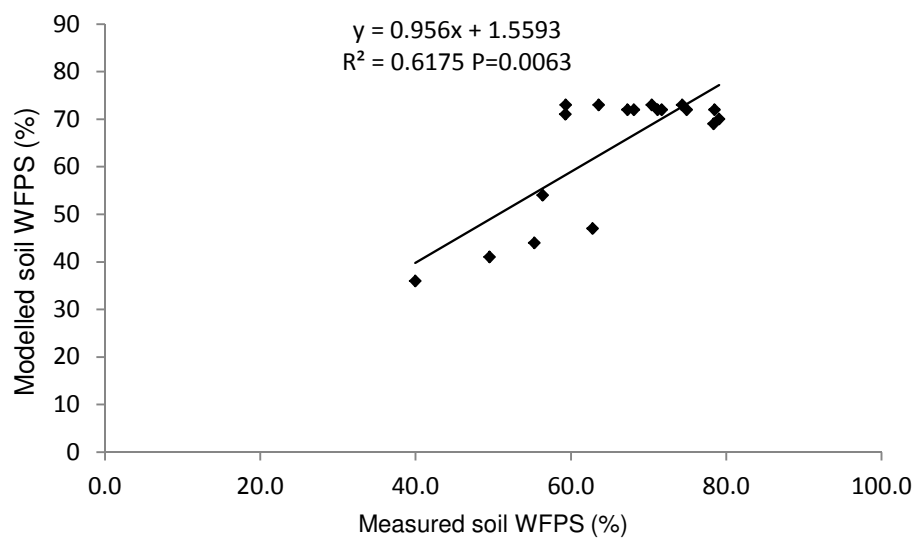


Figure D1 UK-DNDC model output for soil WFPS at a) 1cm depth and b) 5 cm depth showing different input values for water retention layer (WRL) depth (cm)

a)



b)

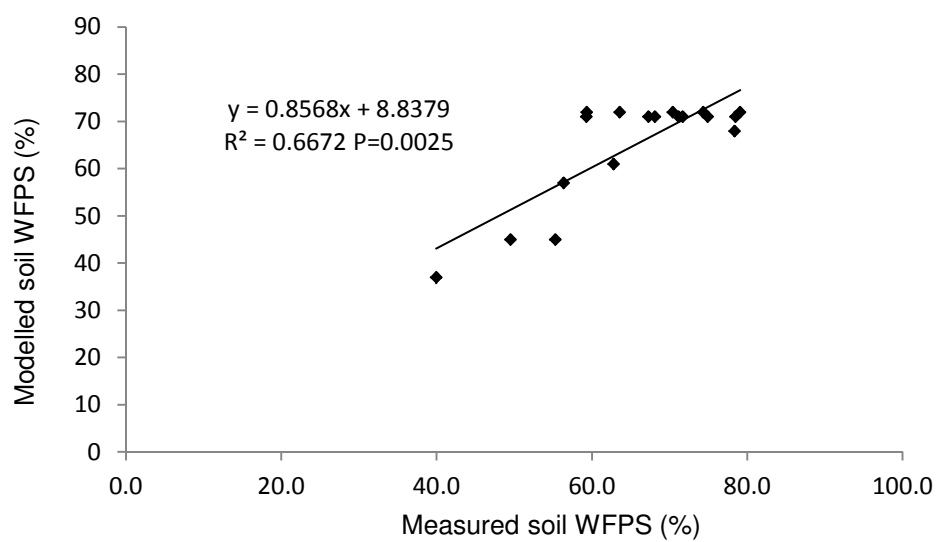


Figure D2 Relationship between modelled and measured soil WFPS, with WRL in the model set at a) 0.302 cm and b) 9.99 cm. The equation of the best fit linear model as well as the R^2 and corresponding P value are shown.

Appendix E: PLFA concentrations

Table E1 Concentration of specific PLFAs in soil samples collected on 5 March 2012 and 11 March 2012 from each of three plots in the IM field and the SRG at North Wyke.

FAME	Concentration ($\mu\text{g g}^{-1}$)											
	05-Mar-12						11-Mar-12					
	Plot1		Plot 2		Plot 3		Plot1		Plot2		Plot3	
	IM	SRG	IM	SRG	IM	SRG	IM	SRG	IM	SRG	IM	SRG
<i>i</i> 14:0	0.07	0.04	0.06	0.04	0.09	0.03	0.09	0.07	0.06	0.04	0.09	0.04
14:0	0.07	0.04	0.07	0.09	0.08	0.03	0.07	0.06	0.07	0.04	0.09	0.04
<i>i</i> 15:0	0.55	0.29	0.56	0.34	0.62	0.24	0.62	0.41	0.51	0.23	0.61	0.26
<i>a</i> 15:0	0.58	0.31	0.55	0.37	0.64	0.27	0.66	0.43	0.51	0.26	0.62	0.29
15:0	0.03	0.03	0.04	0.03	0.05	0.02	0.06	0.04	0.04	0.02	0.05	0.03
<i>i</i> 16:0	0.22	0.13	0.26	0.15	0.25	0.10	0.25	0.17	0.24	0.09	0.26	0.12
16:0	0.99	0.72	1.04	0.76	1.10	0.57	1.21	1.02	1.21	0.58	1.23	0.67
16:1 ω 9	0.62	0.40	0.64	0.39	0.70	0.29	0.76	0.59	0.71	0.30	0.76	0.36
16:1 ω 7c/t	0.62	0.40	0.64	0.39	0.70	0.29	0.41	0.59	0.71	0.30	0.76	0.36
<i>i</i> 17:0	0.38	0.50	0.41	0.22	0.67	0.42	0.24	0.67	1.06	0.40	1.00	0.47
<i>a</i> 17:0	0.23	0.11	0.24	0.13	0.24	0.09	0.50	0.14	0.22	0.08	0.23	0.10
<i>n</i> 17:0	0.06	0.03	0.06	0.04	0.07	0.03	0.04	0.04	0.06	0.03	0.06	0.03
<i>cy</i> 17:0	0.79	0.40	0.80	0.44	0.82	0.32	0.85	0.37	0.78	0.28	0.81	0.24
18:0	0.61	0.35	0.73	0.43	0.68	0.30	0.70	0.46	0.70	0.28	0.69	0.31
18:1	0.19	0.14	0.12	0.15	0.22	0.11	0.25	0.17	0.26	0.11	0.22	0.12
C18:1 ω 9	0.81	0.50	1.53	0.60	0.94	0.44	0.99	0.46	1.26	0.47	0.95	0.50
C18:1 ω 7	1.45	0.84	0.27	0.91	1.53	0.68	1.78	1.21	1.78	0.73	1.56	0.77
10-Me 18:0	0.20	0.09	0.27	0.13	0.21	0.08	0.23	0.10	0.24	0.07	0.23	0.11
18:2 ω 6	0.13	0.16	0.18	0.22	0.21	0.19	0.31	0.29	0.39	0.21	0.28	0.25
<i>cy</i> 19:0	0.85	0.42	0.73	0.47	0.81	0.35	0.98	0.55	0.86	0.29	0.78	0.32

Appendices

Table E2 E1 Concentration of specific FAMES in soil samples collected on 19 April 2012 and 17 May 2012 from each of three plots in the IM field and the SRG at North Wyke.

FAME	Concentration ($\mu\text{g g}^{-1}$)											
	19-Apr-12						17-May-12					
	Plot 1		Plot 2		Plot 3		Plot 1		Plot 2		Plot 3	
	IM	SRG	IM	SRG	IM	SRG	IM	SRG	IM	SRG	IM	SRG
<i>i</i> 14:0	0.08	0.06	0.09	0.05	0.10	0.04	0.05	0.04	0.07	0.04	0.06	0.04
14:0	0.09	0.06	0.09	0.06	0.09	0.04	0.04	0.04	0.07	0.06	0.06	0.04
<i>i</i> 15:0	0.62	0.38	0.59	0.41	0.63	0.29	0.26	0.28	0.51	0.26	0.03	0.22
<i>a</i> 15:0	0.60	0.41	0.57	0.44	0.63	0.31	0.26	0.28	0.51	0.27	0.44	0.24
15:0	0.05	0.05	0.05	0.05	0.06	0.03	0.03	0.03	0.04	0.03	0.04	0.02
<i>i</i> 16:0	0.29	0.18	0.27	0.20	0.29	0.15	0.15	0.15	0.26	0.14	0.24	0.12
16:0	1.13	0.99	1.14	1.08	1.24	0.82	0.03	0.79	1.16	0.83	1.08	0.75
16:1 ω 9	0.69	0.07	0.65	0.48	0.78	0.42	0.05	0.05	0.68	0.37	0.60	0.03
16:1w7c/t	0.69	0.54	0.65	0.48	0.78	0.42	0.35	0.37	0.68	0.37	0.60	0.30
<i>i</i> 17:0	0.99	0.61	0.96	0.65	1.09	0.51	0.50	0.19	1.04	0.47	0.88	0.38
<i>a</i> 17:0	0.26	0.14	0.23	0.16	0.25	0.12	0.12	0.12	0.24	0.12	0.30	0.09
<i>n</i> 17:0	0.06	0.00	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.06	0.04
<i>cy</i> 17:0	0.77	0.39	0.70	0.35	0.84	0.29	0.35	0.38	0.74	0.36	0.70	0.27
18:0	0.70	0.40	0.70	0.48	0.72	0.38	0.55	0.00	0.57	0.39	2.46	0.28
18:1	0.21	0.14	0.24	0.16	0.25	0.14	0.12	0.15	0.29	0.39	0.25	0.09
C18:1 ω 9	0.64	0.48	0.97	0.52	1.03	0.49	0.58	0.54	1.22	0.72	1.17	0.46
C18:1 ω 7	1.12	0.85	1.47	0.75	1.73	0.72	1.01	0.89	1.90	0.88	1.67	0.63
10-Me 18:0	0.25	0.12	0.26	0.18	0.23	0.15	0.18	0.12	0.31	0.12	0.28	0.11
18:2 ω 6	0.30	0.42	0.05	0.52	0.34	0.35	0.42	0.24	0.40	0.16	0.48	0.41
<i>cy</i> 19:0	0.42	0.24	0.68	0.26	0.82	0.19	0.35	0.40	0.81	0.34	0.67	0.21

Appendix F Review paper submitted to Land Use Policy

Title: Is extensification a viable alternative for enhanced provision of ecosystems services in UK agriculture?

Abstract

Extensification is offered as a management strategy to reduce the perceived negative impacts of intensive agricultural management, i.e. nutrient losses through leaching and greenhouse gas emissions, declining soil and water quality, and reduced biodiversity. Initially, intensively managed land use types (arable and grassland) are taken out of production for either five, seven or ten years in the UK. The ultimate objective is the establishment of species-rich grassland (SRG) which receives limited or no fertiliser, and attracts subsidy payments under the European Agricultural Fund for Rural Development. There is little scientific evidence for the anticipated environmental benefits of these newly-created SRGs. The legacy of intensive management on soil is likely to limit ES provision from former intensively managed sites for many decades following extensification. Clearly, programmes of long-term experimental research need to be implemented to quantify the environmental and economic benefits of current extensification schemes in UK agriculture in order to determine whether the benefits of their implementation out-weigh the costs in terms of loss of production.

Key words

Ecosystem Services; Agr-environment scheme; sustainable agriculture; grassland; nutrient cycling.

1. Policy underpinning extensification in UK agricultural soils

Soils and land use play a central role in the delivery of supporting, provisioning, regulating and cultural Ecosystem Services (ES) in the UK (see review by Haygarth and Ritz, 2009). Agri-environment schemes aimed at enhancing the environmental value of land are funded under Axis 2 of the European Agricultural Fund for Rural Development (EAFRD) and include the extensification of previously intensively managed agricultural land (arable and grassland) by limiting or ceasing fertiliser applications, often with subsequent re-sowing of prescribed species-rich grassland seed mixes to create semi-natural species-rich grasslands (EC, 2009). Agri-environment schemes were first introduced in the UK in 1985 (Smith et al., 2008), with the more targeted, pro-active approach to restoring habitats such as species-rich grasslands (SRGs) beginning with the introduction of environmental stewardship. Land managers receive funding through a variety of initiatives to manage SRGs according to scheme guidelines (see Table 1). It is envisaged that implementation of SRGs will enhance delivery of ES of financial benefit to farmers as well as wider benefits for human health and wellbeing (Millennium Ecosystem Assessment, 2005). With the current UK Rural Development Programmes due for review for the 2014-2020 period, now is the time to assess the benefit of currently funded schemes.

2. Potential benefits of extensification.

Long term extensively managed grasslands provide multiple ES including: improved carbon and nitrogen retention (Culman et al., 2010); reduced nitrate leaching (de Vries et al., 2011); and biodiversity provision (Janssens et al., 1998). These benefits are perceived to apply to newly-created or restored extensive grasslands, but a lack of long term data exists to support these claims. This review assesses the possible impact of extensification on nutrient cycling, water quality, greenhouse gas emissions and biodiversity. It will consolidate information from published data, highlight how the benefits of current grassland creation (on former arable land) and restoration (on former intensive grassland) schemes could be limited, and recommend further research to answer remaining uncertainties (Fig1).

3. Does extensification improve nutrient cycling efficiency?

3.1 *A time lag in microbial response can reduce nutrient cycling efficiency*

When exposed to a stress, such as the shift in management associated with entry into an agri-environment scheme (Eisenhauer et al., 2010), ecosystems can initially display characteristics of early successional systems, with reduced efficiency of internal nutrient cycling and increased losses (Odum, 1985). Soil microbes play a key role in nutrient cycling (Bardgett, 2005), and there can be a time lag of several decades between a land-use change and the microbial community resembling that of the new system (Buckley and Schmidt, 2001). This time lag has implications for nutrient cycling; for example, following extensification the soil fungal:bacterial ratio increases (Bardgett and McAlister, 1999; van der Wal et al., 2006), but it can take many decades before the ratio resembles that of a the target grassland ecosystem (van der Wal et al., 2006; Maharning et al., 2009). Rates of nutrient cycling (van der Heijden et al., 2008) and N losses (de Vries et al., 2011) tend to be greater in bacterial-dominated soils.

Long-established grasslands typically have a complex soil food webs (Culman et al., 2010), with competition (Stienstra, et al., 1994) and plant-soil feedbacks (Habekost et al., 2008, Bardgett et al., 2009) encouraging nutrient cycling efficiency. Productive crops and grasses, supported by intensive agriculture, rapidly take up nutrients from the soil, whilst plant species sown and encouraged by the creation of SRG are adapted to nutrient-poor environments and have a low nutrient uptake capacity (Hobbie, 1992; Aerts and Chapin, 2000;); they decrease their uptake of P (Breeze and Hopper, 1987) and N (Glass et al., 2002; Siddiqi et al., 1990) when tissue concentrations are sufficient. The response of the soil biota often lags behind plant species change, delaying the establishing of efficient plant soil feedbacks and nutrient cycling (Holtkamp et al., 2008; Eisenhauer et al., 2010.). At one set of arable plots, abandoned between 2 and 22 years ago, the soil microbiota showed slow or limited progression towards the natural heathland community composition. N and C mineralisation increased in the abandoned plots but the later successional plant species were unable to utilise the increase in labile mineral N (Holtkamp et al., 2011), which are vulnerable to loss (Haynes and Naidu, 1998).

3.2 *Legacy effects of agriculture on soil C:N ratio can maintain high soil mineral N*

Appendices

The soil C:N ratio can affect the rate of microbial metabolic processes involved in the N cycle. Nitrogen immobilisation is enhanced by a greater soil C:N ratio (Barrett and Burke, 2000). The legacy of intensive agriculture on the soil C:N ratio can continue to impact N cycling for many years after agricultural abandonment. For instance, nitrification rates in a secondary forest were greater than in an adjacent, never-cultivated forest 50 years after agricultural abandonment due to a lower soil C:N ratio (Compton and Boone, 2000). In another forested site, not cultivated since for 2000 years the soil C:N ratio was lower and the rates of net N mineralization greater where historical cultivation and manure application had been the most intense (Dupouey et al., 2002). Therefore, the legacy effects on the soil C:N ratio of former intensive agricultural management in restored grasslands could similarly limit N retention potential for substantial periods.

3.3 Reductions in net nitrification have been recorded at recently restored grasslands although significant changes take over 40 years

Extensification has been shown to reduce nitrification and potential N losses, although many years may be required before rates are similar to those of the natural grassland ecosystems. One chronosequence of restored grasslands, which had not received fertiliser for 3, 7, 20 and 46 years, showed an exponential decline in potential nitrifying activities with increasing years without N fertilisation. The number of ammonium oxidising bacteria only showed a significant decline in the fields that had not received fertiliser for 20 and 46 years (Stienstra et al., 1994). Formerly cultivated plots in prairie grasslands with low soil C and N (McLauchlan, 2006a) showed a decrease in potential net N mineralisation as C and N stocks accumulated over 40 years (McLauchlan, 2006b). However, nitrification rates after 2 years in grasslands on former arable land were similar to those of long-term unfertilized sites, and significantly lower than sites remaining under arable production. (Malý et al., 2000).

3.4 The potentially idiosyncratic response of the soil microorganisms and subsequent ecosystem function, could lead to uncertainty in predicting ES provision

Appendices

Extensification represents a sudden shift in selective stress, which will affect the size and composition of the soil microbial community (Nielsen et al., 2011). The response of soil microorganisms to changes in plant diversity can be unexpected, with individual plant species proving more important in determining microbial community development than plant diversity *per se* (Viketoft, 2008; Eisenhauer et al., 2010).

The nutrient cycling response to changes in the soil biota can also be unpredictable, as only certain species can carry out some steps within nutrient cycles (Nielsen et al., 2011; Naeem et al., 2002).

4. Does extensification cause an improvement in water quality?

4.1 Legacy soil N and P remain a source of potential pollutants to surface water and groundwater

High residual soil fertility (N and P; Walker et al., 2004; Pywell et al., 2007), can persist for many decades following extensification. Elevated soil nutrient concentrations have been recorded in woodlands on former agricultural sites for up to 80 years after the cessation of intensive agriculture (Falkengren-Grerup et al., 2006; Kopecký and Vojta, 2009). Soil N and P concentrations in previously manured plots remained elevated compared to non-fertilised control plots after 16 years, even under continued cultivation. Models suggested it would take 17-41 years for the total N concentration in the surface soil to reach pre-manure-application levels (Indraratne et al., 2009).

Legacy soil P can remain a source of nutrient pollution to surface waters (Scott et al., 2001); adsorbed inorganic P (Pi) can gradually desorb and enter soil solution (Vu et al., 2010), whilst soil erosion can transport significant quantities of particulate P to surface waters, particularly during high energy rainfall events (Kleinman et al., 2011; Dungait et al., 2012). Additionally, grassland ecosystems can be a significant source of P loss to watersheds, through the transfer of fine colloids, even in the absence of high intensity rainfall (Bilotta et al., 2007). Former intensively managed sites may be particularly vulnerable to erosion due to the legacy of management on soil structure, which can remain weakened for many years following extensification (Horn et al., 1995; Baer et al., 2002).

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As with P, accumulated soil N can enter surface waters through erosion. Organic N can also be a source for N loss in solution; it comprises 40-50% of the total soluble soil N pool in English arable soils (Murphy et al., 2000). A review of 16 agricultural field studies showed that dissolved organic N (DON) accounted for a mean of 26% annual soluble N mass loss in leachate (van Kessel et al., 2009). Once leached, DON can have significant ecological impacts and adversely affect the ecology of rivers and estuaries (Seitzinger and Sanders 1997; Vitousek et al., 1997). Thus the agricultural legacy on soil N and P concentrations may limit the benefit of extensification for improving water quality.

4.2 Improvements in river ecology can lag behind management changes

The benefits of extensification on riverine ecology may not be observed for decades. The causes of this time lag include long residence times for groundwater that contains legacy nutrients that to feed streams, and earlier nutrient accumulation in stream bed and flood plain sediments (Hamilton, 2012).

4.3 Rates of nitrate leaching can decrease but the scale of decline is limited by the presence of legumes and low plant species diversity

Legumes increase total N availability through N₂ fixation and decrease the C:N ratio of plant litter so decreasing N immobilisation and leaching. A directly proportional relationship between rates of mineral N leaching and number of legumes present in grassland swards has been suggested (van Kessel et al., 2009). Nitrate leaching is low in recently established grasslands on former arable sites where legumes are absent and plant species diversity high, thus, increasing biodiversity in legume-rich swards reduces N leaching by increasing productivity and N uptake (Scherer-Lorenzen et al., 2003). However, the level of intervention required to establish and maintain high species diversity is unfeasible for most agri-environment schemes.

5. Does extensification lead to a decrease in greenhouse gas emissions?

5.1 The likely response of nitrous oxide fluxes to the combined effect of management change on multiple soil properties is uncertain

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Agriculture accounts for 63% of the EU's nitrous oxide (N₂O) emissions (Johnson et al., 2007). Nitrous oxide is a potent greenhouse gas (GHG) with a climate forcing effect 298 times that of CO₂ (IPCC, 2007). N₂O is released from soils as a product of denitrification (van Groenigen et al., 2010) which is promoted by high soil mineral N concentrations (Baggs et al., 2000), an active community of denitrifying bacteria (Cavigelli and Robertson, 2000; Morales et al., 2010) and increased soil temperature and water filled pore space (WFPS) (Smith et al., 1998b). Thus, the response of these soil properties to extensification will regulate emissions of this important GHG.

Soil temperature and WFPS influence N₂O emissions from soils, but how these soil properties will be influenced by extensification is not clear (Orwin et al., 2010). Soil WFPS increases more rapidly following rainfall in arable soils with higher bulk densities leading to increased N₂O emissions (Rochette, 2008). Canopy structure affects heat transmission to the soil surface (Peacock, 1975), whilst the increased vegetation density of tussocky grass swards can reduce water evaporation following peak rainfall, ensuring soil WFPS remains elevated for longer (Caldeira et al., 2001). Also, highly productive species tend to have a higher transpiration rates (Blum, 2005). Thus, encouraging the establishment of a mosaic of plant species of different functional types could decrease evapotranspiration maintaining higher soil WFPS.

5.2 Grassland soils can sequester carbon, although this may not represent a true decrease in total atmospheric GHGs

The creation of grasslands on former arable land (Conant et al., 2001) and changes in management of existing grassland (De Deyn et al., 2011) can enhance soil C sequestration. However, management changes that lead to increased soil C stocks may cause an increase in the emissions of non-CO₂ GHGs (Powlson et al., 2011) and may require N addition from either from legumes or fertiliser (Conant et al., 2001) which have associated environmental costs. Furthermore, the temporary creation of grassland for periods of 5–10 years, as encouraged under current agri-environment schemes, can only support limited and temporary increases in soil C stocks; even after 50 years carbon stocks in some restored grasslands have not reached pre-agricultural levels, whereas, within six years of resuming cultivation, soil C stocks can be severely depleted (Soussana et al., 2004).

6. Does extensification enhance plant diversity?

6.1 Legacy N and P can limit the diversity and conservation value of plant species in grasslands on former intensively managed land

The most diverse grasslands with plant species of the highest conservation value tend to occur on nutrient poor soils (Critchley et al., 2002). Thus, high concentrations of legacy soil N and P, which can persist for decades following extensification, can limit the biodiversity value of created or restored grasslands (Walker et al., 2004). The effects of N addition on plant species composition were still apparent 15 years after fertiliser application ceased at one UK grassland site, with previously fertilised sites dominated by faster growing species, typical of nutrient rich environments. Recovery of the grassland was slowest at sites which had received the highest rates of historical fertiliser application (Stevens et al., 2012).

6.2 Some diverse grasslands have been successfully created / restored within 20 years, however attempts to help enhance biodiversity can have adverse effects on other ES

Some agri-environment schemes have proved successful at re-establishing diverse plant communities in a relatively short time. A recent review of grassland and heathland creation schemes in England identified 62 grassland sites that met the criteria for designation as priority habitat, according to the UK biodiversity action plan. Most of these grasslands had been established for 8-15 years and a combination of seed drilling, hay application and natural regeneration had been used (Wilson et al., 2013).

Several studies have investigated management techniques to improve the success of grassland restoration schemes in restoring high plant diversity. Smith et al. (2008) found that the most effective regime for restoring upland hay meadows was complete cessation of fertiliser application, cutting in July and grazing with cattle in autumn and sheep in spring. However even with this regime it was suggested that 20 years would be required for the target community to establish. A recent review of grassland restoration techniques discusses the merits of using different seed sources and application methods. The sowing of a high-diversity, locally-sourced seed mixes

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at a high densities can enable more rapid biodiversity restoration, but the cost of this method is high, which may prohibit their use by agri-environment scheme entrants unless subsidy payments are increased (Török et al., 2011). Soil preparation by removal of topsoil, and ploughing or harrowing so that seeds can be sown on bare ground is a commonly used restoration technique that can reduce the time taken to achieve the desired diversity (Pywell et al., 2007). However, these methods leave soil vulnerable to erosion and leaching (Vitousek and Reiners, 1975), so, whilst their effect on biodiversity provision may be positive they encourage nutrient losses which are both environmentally harmful and wasteful.

7. Trade-offs with intensive ES provision from intensive managed sites

7.1 The loss of production following extensification must be balanced by gains in other ES

Intensive agriculture can have detrimental effects on environmental ES, particularly when fertiliser applications are poorly timed or in excess of requirements (Dungait et al., 2012). Hence, at first glance, extensification schemes, whereby fertiliser additions are greatly reduced or stopped altogether, may seem an effective way to reduce these effects and benefit ES provision. However, removing land from intensive agriculture greatly reduces the food production potential. Therefore, it is vital that this loss in an essential ES is balanced by gains in other ES, including reductions in GHG emissions, improved nutrient cycling efficiency and water quality and enhanced biodiversity provision.

8. Conclusions and Recommendations

This review suggests that the potential for newly-created or restored SRGs to provide ES, other than food production, may be severely limited for the first few decades after creation. In addition, continued improvements in our understanding of nutrient cycles (Dungait et al., 2012), coupled with evolving management techniques, aim to maintain production whilst minimising environmental damage from land remaining under intensive management. Our understanding of the potential for the legacy of intensive agriculture to limit ES provision by grasslands created under agri-environment schemes is limited by a lack of long term studies (Sutherland, 2004).

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Much existing data on the legacy of intensive agriculture on soil properties and functioning has been obtained from chronosequences of abandoned farmland, former agricultural sites that are now forested, and agricultural sites that were abandoned prior to the introduction of synthetic fertilisers (Macdonald et al., 2012).

Furthermore, despite calls to assess the impact of management change on multiple ES delivery (Bennett and Balvanera, 2007), evaluations of grassland creation / restoration schemes tend to focus on the provision of a single ES, usually plant diversity (DARD, 2008; Wilson et al., 2013). Long term measurement of GHG emissions, including N₂O and CO₂, and nutrient leaching, particularly P and organic N, from agri-environment schemes are missing from the literature. We suggest this knowledge gap is addressed as a matter of urgency, particularly in light of a growing interest in payments for ecosystem services as a method for maximising the environmental value of land (Kroeger and Casey, 2007).

Acknowledgements

We thank Roland Bol and Emma Pilgrim for providing advice and proof reading of early drafts. We also acknowledge financial support from the Natural Environment Research Council (NERC) the and the Scottish Environment Protection Agency (SEPA). This work represents part of the BBSRC-funded programmes at Rothamsted Research on Sustainable Soil Function and Climate Change.

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